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BEARING ON HEREDITY AND VARIATION

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MUTATIONS INVOLVING THE PRODUCTION OF CONIDIA AND THE REQUIREMENT FOR LEUCINE IN A MUTANT OF NEUROSPORA

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Received January 22, 1948

AMONG the mutants of *Neurospora crassa* are some which require leucine for growth (REGNERY 1947). Two of these, strains 8839 and 33757, are completely unable to synthesize leucine and both behave in crosses as though this were due to differences from "wild-type" in single genes. Strain 8839 was secured after X-radiation, strain 33757 after ultraviolet irradiation. Both were probably induced mutations, for 0.7 percent mutants were secured by the

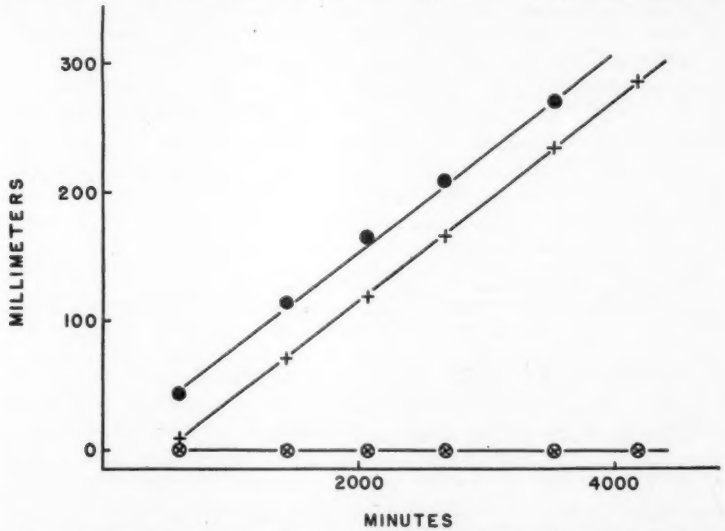

FIGURE 1.—The growth rates of "wild-type" *Neurospora crassa* Nr. 1 (black dots), a heterocaryon between the two leucineless strains 8839-4637-A and 33757-4637-A (pluses) and the separate components (open circles and crosses) on medium devoid of leucine.

treatment which preceded the isolation of 8839 and 0.5 percent after the treatment preceding the isolation of 33757 (BEADLE and TATUM 1945). On the other hand, the spontaneous incidence of biochemical mutants appears to be about 0.1 percent (HOROWITZ *et al.* 1946).

Neither strain 8839 nor strain 33757 will progress by itself on the surface of agar in the absence of leucine. However, when they are brought together in a

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heterocaryon the rate of progression on the surface of agar in the absence of leucine is equal to that of the "wild-type" mold (fig. 1). This is presumptive evidence that the genes determining the requirement for leucine are not alleles (BEADLE and COONRADT 1944). The mutant locus in strain 8839 can be designated l_2 while that in strain 33757 is called l_1.[1]

When these strains are crossed together the leucineless character segregates as though it were determined in the two cases by independent genes. Of the

TABLE 1

The segregation of leucineless factors in a cross of leucineless strain 8839-4637-A with leucineless strain 33757-a ($l_2L_1 \times L_2l_1$).

	TYPE 1 ASCUS				TYPE 2 ASCUS				TYPE 3 ASCUS			
SPORE NO.	GROWTH ON MEDIUM DEVOID OF LEUCINE	GROWTH ON MEDIUM DEVOID OF LEUCINE BY A HETEROCARYON FORMED WITH STRAIN		GENOTYPE	GROWTH ON MEDIUM DEVOID OF LEUCINE	GROWTH ON MEDIUM DEVOID OF LEUCINE BY A HETEROCARYON FORMED WITH STRAIN		GENOTYPE	GROWTH ON MEDIUM DEVOID OF LEUCINE	GROWTH ON MEDIUM DEVOID OF LEUCINE BY A HETEROCARYON FORMED WITH STRAIN		GENOTYPE
		8839	33757			8839	33757			8839	3357	
1	−	+	−	l_1L_2	−	−	−	l_1l_2	−	−	−	l_1l_2
2	−	+	−	l_1L_2	−	−	−	l_1l_2	−	−	−	l_1l_2
3	−	+	−	l_1L_2	−	+	−	l_1L_2	−	−	−	l_1l_2
4	−	+	−	l_1L_2	−	+	−	l_1L_2	−	−	−	l_1l_2
5	−	−	+	L_1l_2	−	−	+	L_1l_2	+			L_1L_2
6	−	−	+	L_1l_2	−	−	+	L_1l_2	+			L_1L_2
7	−	−	+	L_1l_2	+			L_1L_2	+			L_1L_2
8	−	−	+	L_1l_2	+			L_1L_2	+			L_1L_2

+ refers to growth, − to failure to grow. All strains grew on medium to which leucine was added. The orders of the spores within the asci differ from those presented depending upon the presence of crossing over and the pattern of segregation.

eight spores in the resulting asci, eight, six or four may, upon germination, give rise to leucineless cultures. If all eight spores are leucineless each spore will carry but one of the mutant genes; if six spores are leucineless one pair of spores will carry both mutant genes. If only four spores are leucineless both of these pairs should carry both of the mutant genes and be double-mutants. The double-mutant spores can be distinguished from single-mutant spores by determining whether cultures derived from them will form vigorously growing heterocaryons with the mutant parents. Single-mutants form heterocaryons with one or the other parent while the double-mutants form heterocaryons with neither. Results of this sort were obtained from a cross of strain 8839-4637-A with strain 33757-a as shown in table 1.

The spores from thirteen complete asci were dissected in order and germinated. In every case the albino mutation (4637) segregated as though it were

[1] Upon the suggestion of PROFESSOR MARCUS RHOADES we propose to call the mutant gene which causes the leucine requirement in strain 33757, l_1 and to call its wild type allele L_1. This mutant gene in strain 8839 would then be called l_2 and its wild type allele L_2. This system of nomenclature replaces that previously used (RYAN and LEDERBERG 1946; RYAN 1946).

due to a difference from "wild-type" in a single locus. Two asci contained only leucineless spores, nine contained six leucineless and two leucine-independent spores each, while two had four leucineless and four leucine-independent spores apiece. From six of these asci those single-spore cultures which proved to be leucineless were tested by heterocaryon formation with strains 33757 and 8839 of both mating types. One ascus which contained only leucineless spores showed the type 1 arrangement of genotypes. The two asci which had only four leucineless spores showed the type 3 arrangement. This indicates that the two factors are probably on different chromosomes, although four strand double crossing over between two linked loci would give the same result. The remaining three asci tested, which contained only six leucineless spores, had the type 2 arrangement of genotypes and were due to crossing over between the l_2-L_2 locus and the centromere. One of the double-mutants from a type 3 ascus was crossed with a "wild-type" strain, 15300-A. Since both mutant types, l_1 and l_2, were recovered among four of the five asci dissected the identification of the double-mutant by the heterocaryon method was confirmed.

Consequently, in these asci from the cross of strains 8839-4637-A and 33757-a, segregation occurred in an orthodox fashion in accordance with the results obtained by REGNERY (1947).

In one unique ascus obtained from this cross, although only four of the spores were leucineless, cultures derived from them formed vigorous heterocaryons with strain 8839. Hence, the leucineless character was due to the presence of the mutant locus l_1. Since the remaining three spores (one spore, the third, failed to germinate) were leucine independent, locus l_2 which should have been introduced by the 8839 parent was unaccounted for. Also segregating in this ascus was a factor which prevents the formation of asexual spores, or macroconidia. This aconidiate character seemed to have arisen *de novo* in the aberrant ascus since it was not exhibited by either parent nor was it known to have been present in the lineage from which they were derived. Yet the fourth spore, C4, which was leucine-independent, and the seventh and eight spores C7 and C8, which were leucineless, gave cultures which exhibited the aconidiate character (table 2).[2] Factors for color and mating type segregated normally in this ascus.

The possibility that this ascus arose through contamination is excluded because until it appeared no aconidiate strain existed in our laboratory. Moreover, contamination with a non-conidiating strain would be difficult to achieve. There remain two major hypotheses which can account for the aberrant ascus. The first explanation assumes that the aconidiate character and the failure of the l_2 factor to express itself are due to a non-Mendelian but heritable change. The second hypothesis assumes that the new characters are due to one or more genic changes inherited in a Mendelian fashion.

[2] The aconidiate leucineless strains showed slight initial growth on agar medium devoid of leucine but within a few centimeters this growth ceased. By testing the growth of mycelial and conidial inocula of the conidiate leucineless strain this initial growth was shown to be a function of the mycelial nature of the inoculum. Apparently mycelial fragments carry over more leucine than do conidia.

In order to discriminate between these two hypotheses a culture derived from spore C4 which was leucine-independent and aconidiate was crossed with the conidiate leucineless strain 8839 of the opposite mating type. Spores from three whole asci were isolated and of the resulting cultures half were leucineless and half leucine-independent. In these asci the conidiate and aconidiate factors also segregated in a 1:1 Mendelian fashion. Moreover, in two of the asci the aconidiate and leucine-independent characters segregated independently. In one of these asci, although both characters showed 1st division segregation, new combinations of aconidiate with leucineless and conidiate with leucine-

TABLE 2

Segregation in the unusual ascus (C) from a cross of 8839-4637-A with 33757-a.

SPORE NO.	COLOR[x]	CONIDIA[y]	MATING TYPE	GROWTH[z] ON MEDIUM DEVOID OF LEUCINE	GROWTH[z] ON MEDIUM DEVOID OF LEUCINE BY A HETEROCARYON FORMED WITH STRAIN	
					8839	33757
C1	−	+	A	+	..	..
C2	−	+	A	+	..	..
C3[w]	..	..	..	..	..	..
C4	−	−	A	+	..	..
C5	+	+	a	−	+	−
C6	+	+	a	−	+	−
C7	+	−	a	−	+	−
C8	+	−	a	−	+	−

[w] This spore did not germinate.
[x] + refers to pink conidial pigment, − to albino.
[y] + refers to presence, − to absence.
[z] + refers to growth, − to failure to grow.
All strains grew on medium to which leucine had been added.

independence were formed. These data suggest that the factors determining the absence of conidia and the ability to carry out the step in the synthesis of leucine that is blocked in strain 8839 are on different chromosomes (although four strand double crossing over between two linked loci would also give this result). The two genes are definitely not alleles. Confirmation of this conclusion comes from the random isolation of spores from a cross of the aconidiate culture derived from spore C7 with the leucineless conidiate strain 8839 of the opposite mating type. Of the 93 cultures derived from these spores, 42 were leucineless and carried gene l_2 and 51 were leucine-independent and appeared to carry gene L_2. This 1:1 ratio ($P=0.35$) indicates a Mendelian segregation. Likewise the aconidiate and conidiate characters segregated 39 to 54 ($P=0.12$ for a 1:1 segregation). Again new combinations between aconidiate and leucineless were found. Thus this evidence also indicates that absence of conidia and the ability to carry out the step in leucine synthesis that is blocked in strain 8839 are determined by different genes.

Of the 93 spores isolated at random, however, 58 were new combinations of the conidiate and leucineless factors while only 35 were old combinations (P = 0.02 for a 1:1 ratio). This excess of new combinations may be explained in several ways. Four strand double crossing over between two linked loci would give this result, but, as in the cases of the whole ascus analyses already mentioned, it would have to be assumed to occur with an unprecedented frequency. Although crossing over may be non-random in Neurospora there is no reason to believe that four strand doubles occur in any excess (LINDEGREN and LINDEGREN 1937, 1939; WHITEHOUSE 1942). Since the discrepancy from a 1:1 ratio of old to new combinations is almost entirely due to the preponderance of

TABLE 3

Tests for linkage of the albino locus (al_1) in strain 4637 with the gene for absence of conidia (ac_1) and the leucineless locus (l_2). Number of cultures carrying old and new combinations.

	l_2 AND al_1	
Cross	8839-4637-A×R978-a (l_2-al_1) (L_2-Al_1)	C7-a×8839-4637-A (L_2-Al_1) (l_2-al_1)
Old Combinations	155	43
New Combinations	142	50
	c_1 AND al_1	
Cross	C4-A×R978-a (c_1-al_1) (C_1-Al_1)	C7-a×8839-4637-A (c_1-Al_1) (C_1-al_1)
Old Combinations	247	75
New Combinations	60	18

the conidiate, leucine-independent or "wild-type" class of spores we are inclined to believe that a differential viability is its cause. The differential viability might be related to the fact that strain 4637, one of the parents of strain 8839-4637-A, is known to contain a chromosomal aberration (MCCLINTOCK 1945). Most probably, as the whole ascus analyses showed, the genes for absence of conidia and independence of leucine (as it is required by strain 8839) are on different chromosomes. This conclusion is borne out but not proved by the linkage relationships of genes l_2 and c_1.

Random isolation of spores from two crosses involving gene l_2 and a gene for albinism, al_1, indicates that the factors are not linked (table 3; P = 0.5 for a 1:1 ratio). On the other hand, as table 3 shows, random isolation of spores from two crosses involving al_1 and the aconidiate gene, c_1, indicates a strong linkage between the two. Gene c_1 lies about 20 map units from the albino gene al_1 and consequently is on the mating type (sex) chromosome (DOERMANN 1944). The mutant gene fluffy, which also results in the failure of conidia production by *Neurospora crassa* is known not to lie on the mating type chromosome (LINDEGREN and LINDEGREN 1939). The non-allelic nature of genes c_1 and

fluffy was proven by making a conidiating heterocaryon between stocks bearing these factors.

Knowing that the aconidiate character in the aberrant ascus is due to a chromosomal mutation, it is still necessary to demonstrate that the ability of *all* of the spores in the aberrant ascus to carry out the step in leucine synthesis that is blocked in strain 8839 is due to a mutation inherited in a Mendelian fashion. Since this type of leucine-independence would be due in half of the spores in the unusual ascus to the L_2 gene introduced by the 33757 parent we do not know which of the spores to suspect as mutant in this regard. It is therefore necessary to examine one member of at least three of the four pairs of spores in the unique ascus. The results achieved after crossing members of two different pairs of spores with strain 8839 have already been mentioned. Cultures from spores C4 and C7 both give 1:1 segregations of dependence and independence of leucine (as it is required by strain 8839). In addition the isolation and characterization of spores from four whole asci produced by a cross of a culture from spore C2 and strain 8839 yielded 1:1 segregations of the leucineless and leucine-independent characters. It follows that the ability to carry out the step in the synthesis of leucine which is blocked in strain 8839, is, in the unusual ascus, inherited in a Mendelian fashion.

This ability, however, may in the two pairs of mutant spores in the unusual ascus be due either to the presence of gene L_2 derived by back-mutation from l_2 or to the presence of a suppressor mutation at some other locus. To distinguish between these two possibilities cultures from spores C2, C4, and C7 were crossed with wild-type strains of the opposite mating type. If a mutation at a locus other than l_1 was responsible for leucine-independence, then, due to a separation of the two loci, some leucineless spores should occur among the progeny. From a cross of strain C2 with strain R978, 91 single-spore cultures were obtained, 307 were secured from a cross of C4 with R978 and 108 from a cross of C7 with 15300. None of these progeny, however, were leucineless at the l_2 locus. Hence, the absence of the expression of gene l_2 among the spores of the unusual ascus is probably due to the back mutation of the l_2 locus to the L_2 condition.

Since all of the spores in the unusual ascus seem to carry L_2, the mutation must have occurred at least as early as the stage of the diploid zygote nucleus. It may have occurred earlier during the vegetative growth of the parent 8839 culture, which would then have been a heterocaryon. This would account for the fact that only one of fourteen asci from the original cross of 8839 and 33757 was unusual. Similarly, the aconidiate gene, c_1, may have existed in a small number of nuclei in one of the parent strains, which became heterocaryotic by spontaneous mutation. LINDEGREN (1942) has pointed out how this can occur when stocks are constantly maintained by conidial transfer. We believe that the mutation C_1 to c_1 occurred in strain 8839-4637-a for the following reasons. First, among the hundreds of spores isolated from crosses of strain 33757 in our laboratory the aconidiate mutation has never appeared. In the second place, since the discovery of the abberant ascus, aconidiate cultures have been secured on numerous occasions from spores obtained by crossing wild-type strains with the same stock of 8839-4637-A which was the parent of the unusual ascus. For

example, among 341 spores isolated from one cross, 44 were aconidiate. Of these 59 percent were leucineless and 41 percent were leucine-independent, indicating again that genes c_1 and l_1 are independent of one another.

If the aconidiate mutation and the back-mutation of l_2 to L_2 both occurred in the 8839-4637-A parent they must have occurred in the same nucleus. We do not feel compelled to believe, however, that they occurred at the same time for the back mutation of l_2 may not be a very rare event. Among 105 cultures of strain 8839-4637-A grown in the presence of limiting amounts of leucine in liquid medium, 9 showed adaptation. By analogy with strain 33657 adaptations are due to back mutations of the leucineless gene (RYAN 1946). REGNERY (1947) has also found strain 8839 to be unstable. It appears most likely that the unusual ascus was due to a back-mutation of the l_2 locus to the L_2 condition in a nucleus which already carried the c_1 mutation.

SUMMARY

Two leucineless strains of *Neurospora crassa*, 8839 and 35757, were crossed to produce a double mutant. In the majority of cases segregation was as would be expected if the two strains were leucineless because of mutations in different genes located on different chromosomes and affecting different steps in the synthesis of leucine.

In one ascus, however, occurred what was apparently a non-Mendelian segregation. Analysis of this ascus indicated that gene l_2 in strain 8839 can mutate back to the "wild-type" condition, L_2. In addition, the line of nuclei carrying the L_2 gene developed an independent mutation to an aconidiate condition. The gene responsible for this condition, c_1, is not linked with l_2 but is on the mating type chromosome.

LITERATURE CITED

BEADLE, G. W., and V. L. COONRADT, 1944 Heterocaryosis in *Neurospora crassa*. Genetics **29**: 291–308.

BEADLE, G. W., and E. L. TATUM, 1945 Neurospora II. Methods of producing and detecting mutations concerned with nutritional requirements. Am. J. Bot. **32**: 678–686.

DOERMANN, A. H., 1944 A lysineless mutant of Neurospora and its inhibition by arginine. Arch. Biochem. **5**: 373–384.

HOROWITZ, N. H., M. B. HOULAHAN, M. G. HUNGATE, and B. WRIGHT, 1946 Mustard gas mutations in Neurospora. Science **104**: 233–234.

LINDEGREN, C. C., 1942 The use of fungi in modern genetical analysis. Iowa State College J. Sci. **16**: 271–290.

LINDEGREN, C. C., and G. LINDEGREN, 1937 Non-random crossing-over in Neurospora. J. Hered. **28**: 105–113.

1939 Non-random crossing over in the second chromosome of *Neurospora crassa*. Genetics **24**: 1–7.

MCCLINTOCK, B., 1945 Neurospora. I. Preliminary observations of the chromosomes of *Neurospora crassa*. Am. J. Bot. **32**: 671–678.

REGNERY, D. C., 1947 A study of the leucineless mutants of *Neurospora crassa*. Dissertation; California Institute of Technology.

RYAN, F. J., 1946 Back-mutation and adaptation of nutritional mutants. Cold Spring Harbor Symposia on Quantitative Biology **11**: 215–226.

RYAN, F. J., and J. LEDERBERG, 1946 Reverse-mutation and adaptation in leucineless Neurospora. Proc. Nat. Acad. Sci. **32**: 163–173.

WHITEHOUSE, H. L. K., 1942 Crossing-over in Neurospora. New Phyt. **41**: 23–62.

A QUANTITATIVE HISTOLOGICAL STUDY OF THE PIGMENT FOUND IN THE COAT COLOR MUTANTS OF THE HOUSE MOUSE. II. ESTIMATES OF THE TOTAL VOLUME OF PIGMENT[1]

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A DETAILED quantitative study of the histology of the pigment granules found in the coat color mutants of the house mouse has been undertaken as a step toward understanding the action of the genes affecting this pigmentation. The first paper of a series giving the results of this study (E. S. RUSSELL 1946) has described the variable attributes of these granules and their condition in each of the genotypes tested. The presentation was entirely analytical: the granules were counted and measured, and their shape, color and distribution recorded. No attempt was made to synthesize the results into a measure of the total amount of melanin produced in each genotype. A strictly analytical approach was maintained as a reaction against one of the chief limitations in earlier quantitative studies of mammalian pigment (DUNN and EINSELE 1938, E. S. RUSSELL, 1939) where the observed differences in weight or volume could not be analyzed further. This difficulty has made it impossible to distinguish qualitatively between any different types of pigment reduction which might result from different genic substitutions. Histological analysis certainly has made such qualitative distinctions possible and in some cases obvious.

Since the writing of the above-mentioned paper, however, it has become clear that some sort of estimate of the total amount of pigment found in each genotype is essential to interpretation of certain types of data. For example, measurements of enzyme content of various genotypes (W. L. RUSSELL 1939, BRAUCH and RUSSELL 1946a) need to be compared with the amounts of pigment present to determine the directness of the relationship between enzyme concentration and pigment production.

There are various possible ways of comparing the total amount of pigment present in various genotypes. Up to the present it has not been possible to obtain the limited comparisons possible with mean color grades determined for each genotype, as no visual series of color grades, such as used by WRIGHT (1915, 1917, 1927) for the guinea pig, has been made for the mouse. Weights of pigment have been determined for some of the sepia genotypes by DUNN and EINSELE (1938). Pigment weight determinations, however, are very difficult to make for the yellow genotypes, as the pigment is very light. A thorough

[1] This work has been aided by grants to the ROSCOE B. JACKSON MEMORIAL LABORATORY from the COMMONWEALTH FUND, ANNA FULLER FUND, JANE COFFIN CHILDS MEMORIAL FUND, and the NATIONAL CANCER INSTITUTE.

[2] FINNEY-HOWELL RESEARCH FELLOW.

study of the amount of pigment in extracts of the yellow genotypes of the mouse has been made by BRAUCH and W. L. RUSSELL (1946b) but no one has as yet been able to find a method which will extract the very insoluble sepia pigments. Thus the quantity of pigment in all mouse color genotypes has never been measured by a single method, and adequate material is not available for the comparisons which must be made for interpretation of gene action.

This second section of the quantitative histological study of mouse hair pigment is an attempt to find a series of comparable values, obtained by the same method for all genotypes, which are proportional to the volume of pigment deposited either in a complete hair or in some particular region thereof. Much of the data quoted is taken directly from the first paper of the series, where the reader may find definition of the terms used and description of the general methods of study. The destruction by fire of W. L. RUSSELL'S stocks of mice carrying the various genetic color combinations upon which all of these studies have been based has made doubly important the immediate publication of the best possible estimate of pigment content from data already available.

METHOD OF ESTIMATING MEAN GRANULE VOLUME

Obtaining an estimate of the total volume of pigment in a hair or portion of a hair from histological data involves finding a value (1) proportional to the total number of granules in that region and multiplying this figure by a value (2) proportional to the mean volume of these granules. While the value proportional to granule number can be determined very easily, the published data on granule size are not so easily adapted to the present need. The size of pigment granules is given in the first paper of this series as the mean greater diameter of 500 granules of each genotype, estimated to the nearest .25μ. There is a wide range of sizes in certain genotypes, and differences in length of single granules which make relatively small differences in the mean when only one diameter is considered become much more important in measures of volume. This is especially true where there are very large pigment bodies, with lengths as much as seven times the mean, and widths proportionately large. Also, there is variation in granule shape both within and between genotypes which makes it impossible to translate measures of length directly to measures of volume.

Therefore, it seemed necessary for estimates of mean granule volume to get a value proportional to the volume of each individual granule by multiplying its length by the square of its width (lw^2). Both diameters were estimated to the nearest .25μ, although this could not be done very accurately as the micrometer scale was divided in 1μ units. As this is a tedious procedure, only 100 granules of each genotype were measured. Accuracy was checked by comparing the mean of the newly determined lengths with the completely independent measurements of the mean greater diameters of 500 granules previously given in paper I (table 1).

Although the newly determined lengths agree fairly well with the more accurate older data, these measurements can not give a perfect picture of granule volume. Since all had to be made on cross-section slides which include

pieces from the 15th to the 45th fields (unit of hair length) only, any variation in granule size near the ends of the hair would not be included in the data (there often is reduction at the apex). Also, the presence of a few very large pigment bodies, called "granular clumps" (E. S. RUSSELL 1946) in certain

TABLE 1

A comparison of two types of determinations of the mean length, in μ, of granules in the coat color mutants of the mouse. The first column gives the mean for each type of the length of 100 granules estimated to the nearest .25μ. The second gives the mean length of 500 granules estimated to the nearest .25μ.

GENOTYPE	NEW (100 GR.) MEAN LENGTH	OLD (500 GR.) MEAN LENGTH S.E.
aaBBCCDDPP	1.41	1.44± .013
aaBBc^{ch}c^{ch}DDPP	1.07	1.05± .010
aaBBc^{e}c^{e}DDPP	0.86	0.94± .014
aabbCCDDPP	0.81	0.77± .007
aabbc^{ch}c^{ch}DDPP	0.80	0.79± .008
aabbc^{e}c^{e}DDPP	0.83	0.77± .012
aaBBCCDDpp	0.66	0.64± .018
aaBBc^{ch}c^{ch}DDpp	0.64	0.62± .015
aaBBCCddPP	1.33	1.23± .011
aaBBc^{ch}c^{ch}ddPP	1.10	1.12± .025
aabbCCDDpp	0.68	0.61± .013
aabbCCddPP	0.90	0.98± .020
aaBBCCddpp	0.69	0.73± .022
aabbCCddpp	0.51	0.67± .023
A^{v}aBBCCDDPP	0.81	0.83± .013
A^{v}ac^{ch}c^{ch}DDPP	0.73	0.77± .009
A^{v}abbCCDDPP	0.81	0.82± .010
A^{v}abbc^{ch}c^{ch}DDPP	0.75	0.76± .010
A^{c}aBBCCDDpp	0.80	0.80± .011
A^{c}aBBCCDDpp	0.80	0.80± .011
A^{v}aBBc^{ch}c^{ch}DDpp	0.71	0.66± .010
A^{v}aBBCCddPP	0.76	0.79± .012
A^{v}aBBc^{ch}c^{ch}ddPP	0.77	0.77± .017
A^{v}abbCCDDpp	0.72	0.79± .011
A^{v}abbCCddPP	0.82	0.82± .015
A^{v}aBBCCddpp	0.85	0.81± .013
A^{v}abbCCddpp	0.92	0.82± .017

genotypes may lead to inaccuracies in estimate of pigment volume. To minimize difficulty from this source, studies of the distribution, frequency and size of clumps were undertaken, using both the cross sections and whole mounts of hairs of each dilute genotype. Although all types were examined, the best measurements of *frequency* of clumps were made in the whole mounts of the dilute yellow genotypes (five in all), where the low total number of pigmented bodies make it possible to count relative numbers of granules and clumps more accurately than in the more heavily pigmented sepias. In these yellows, clump frequency was determined in both awl and zigzag hairs at 10-field in-

tervals. Great irregularity of distribution was found both within and between hairs. The estimates obtained by this method for the ratio of clumps to total pigment bodies varied from 0.8 percent to 1.5 percent, with a mean of 1.1 percent. The most accurate measurements of mean clump *volume* were made from the cross-section slides. The length and width of 100 clumps from each dilute genotype was determined to the nearest micron. Unfortunately, both the slides and the measurements of six of these ten types were lost by fire, but as the value proportional to the mean volume of the clumps of the four remaining

TABLE 2

Values proportional to the mean size of pigmented bodies in dilute genotypes, where both small granules and large clumps contribute appreciably to total pigment. The first column gives the means of length×squared width of 100 granules (volume under 5μ³) of each dilute genotype. In the second column this value is multiplied by 98.9 percent, the mean proportion of granules among total pigmented bodies. The third column gives the means of length×squared width of 100 clumps (volume over 5μ³) of each dilute genotype. In the fourth column this value is multiplied by 1.1 percent, the mean proportion of clumps among total pigmented bodies. The final column gives the value proportional to mean volume of all pigmented bodies, that is, column 2 plus column 4.

GENOTYPE	GRANULE lw^2/n S.E.	×98.9%	CLUMP lw^3/n	×1.1%	TOTAL
aaBBCCddPP	1.63± .13	1.61	58.2	.64	2.25
aaBBc^{ch}c^{ch}ddPP	0.91± .06	0.90	54.5	.60	1.51
aabbCCddPP	0.79± .04	0.78	40.5	.45	1.23
aaBBCCddpp	0.31± .07	0.31	(50)	.55	0.86
aabbCCddpp	0.16± .04	0.16	(50)	.55	0.71
A^{v}aBBCCddPP	0.51± .05	0.50	41.6	.46	0.96
A^{v}aBBc^{ch}c^{ch}ddPP	0.58± .08	0.57	(50)	.55	1.12
A^{v}abbCCddPP	0.62± .03	0.61	(50)	.55	1.16
A^{v}aBBCCddpp	0.72± .06	0.71	(50)	.55	1.26
A^{v}abbCCddpp	0.56± .05	0.55	(50)	.55	1.10

dilute types ranged only from 41.6 to 58.2 cubic microns, it seems safe to estimate the other values as 50 (see table 2). In all dilute genotypes, the volume value upon which estimates of total pigment are based is determined by combining the mean size and frequency of both clumps and granules (mean granule volume×98.9 percent plus mean clump volume×1.1 percent, table 2).

Thus a method has been developed by which it is possible to measure reasonably satisfactorily the mean volume of the pigment granules in all of the coat color mutants. The data have been recorded as the mean and standard error of a value proportional to the mean volume of individual granules (lw^2/n) of each of the genotypes (table 3).

TOTAL VOLUME OF PIGMENT DEPOSITED IN THE HAIR OF THE VARIOUS GENOTYPES

The mean volume data can be used with measures of granule number to determine the total volume of pigment in each genotype. In some cases the

TABLE 3

Values proportional to the amount of pigment produced in full-grown hairs of each of the genotypes tested. The first column gives a value proportional to the mean volume of individual granules of each type (lw^2/n). The second column gives the sum of all counts of the cortical granules, and is thus proportional to the total number of cortical granules deposited. The third is the sum of all counts of medullary granules and is proportional to the total number of medullary granules deposited. Multiplying the sum of the second and third column figures by the mean granule volume value gives column 4, the estimate of a value proportional to the total volume of pigment in a full-grown hair. Multiplying the third column only by the mean volume value gives column 5, a value proportional to the total volume of pigment in the medulla.

GENOTYPE	lw^2/n S.E.	C	M	$(lw^2/n)(C+M)$	$(lw^2/n(M)$
aaBBCCDDPP	1.53± .05	359	512	1332	783
aaBBc^{ch}c^{ch}DDPP	0.79± .06	374	506	695	400
aaBBc^{e}c^{e}DDPP	0.64± .06	22	208	147	133
aaBBc^{e}c^{e}DDPP		0	0	0	0
aabbCCDDPP	0.63± .06	258	504	480	318
aabbc^{ch}c^{ch}DDPP	0.62± .03	206	489	431	303
aabbc^{e}c^{e}DDPP	0.62± .05	34	223	159	138
aaBBCCDDpp	0.38± .08	37	344	145	130
aaBBc^{ch}c^{ch}DDpp	0.37± .12	0	218	81	81
aaBBc^{e}c^{e}DDpp		0	0	0	0
aaBBCCddPP	2.25	96	369	1046	830
aaBBc^{ch}c^{ch}ddPP	1.51	189	391	876	590
aabbCCDDpp	0.33± .06	29	290	105	96
aabbCCddPP	1.23	88	292	467	359
aaBBCCddpp	0.86	2	249	216	214
aabbCCddpp	0.71	0	224	159	159
A^{v}aBBCCDDPP	0.63± .06	54	240	185	151
A^{v}aBBc^{ch}c^{ch}DDPP	0.55± .05	25	149	96	82
A^{v}abbCCDDPP	0.61± .10	56	224	171	137
A^{v}abbc^{ch}c^{ch}DDPP	0.51± .07	23	128	77	65
A^{vv}aBBCCDDpp	0.66± .08	19	234	167	154
A^{v}aBBc^{ch}c^{ch}DDpp	0.41± .04	0	114	47	47
A^{v}aBBCCddPP	0.96	11	209	211	201
A^{v}aBBc^{ch}c^{ch}ddPP	1.12	5	81	96	91
A^{v}abbCCDDpp	0.45± .03	18	217	106	98
A^{v}abbCCddPP	1.16	5	180	215	209
A^{v}aBBCCddpp	1.26	0	223	281	281
A^{v}abbCCddpp	1.10	0	227	250	250

desired value may be the total amount produced throughout a complete growth cycle of the hair follicle, that is, the amount deposited in an entire hair. In others it may be more helpful to know what is deposited during a certain stage of hair growth, or in a certain region of the hair, such as cortex or medulla. By making the proper combination, of the data given in the first paper of this series on the number of granules deposited at successive levels along the hair shaft in both cortex and medulla, with these present data on mean granule volume, it should be possible to obtain the value desired for any purpose. As examples, it may be useful to give the values proportional to the total amount

produced throughout the entire growth cycle (table 3, column 5), the total amount deposited in the medulla of the hair (table 3, column 6), and the amount produced by the hair follicle while the mid-region of the hair (20th to 40th fields) is growing (table 4).

DISCUSSION

Each of the three series of values given in the preceding section on the total volume of pigment in each genotype may be of service in some particular type

TABLE 4

Values proportional to the amount of pigment deposited in the mid-region of the hair of each of the genotypes tested. Left is the non-agouti series, right the yellow. The figures represent the product of the names of length×squared width (lw^2/n) of the granules, multiplied by M_2, the sum of the counts of number of granules per medullary cell at the mid region (mid), that is, the 20th, 30th and 40th fields.

BACKGROUND GENOTYPE	M_2 mid	NON-AGOUTI (lw^2/n) (M_2)	M_2 mid	YELLOW (lw^2/n) (M_2)
BBCCDDPP	271	415	129	81
BBc^{ch}c^{ch}DDPP	258	204	89	49
BBc^{c}c^{c}DDPP	118	76		
bbCCDDPP	270	170	117	71
bbc^{ch}c^{ch}DDPP	258	160	72	37
bbc^{c}c^{c}DDPP	136	84		
BBCCDDpp	184	70	121	80
BBc^{ch}c^{ch}DDpp	114	42	62	25
BBCCddPP	201	452	120	115
BBc^{ch}c^{ch}ddPP	216	326	47	53
bbCCDDpp	150	50	113	51
bbCCddPP	152	187	93	108
BBCCddpp	132	114	124	156
bbCCddpp	122	87	120	132

of investigation. The first series, giving the total volume of pigment in full-grown hairs, should be especially useful for comparison with extractions of pigment, as all of the pigment, both cortical and the medullary, would be present in the extract. There is one limitation to the use of this series, however, which also limits extract work: minor modifiers and/or environmental variations appear to influence cortical pigment more than medullary, which complicates evaluation of the effects of major genic substitutions.

Thus the second series, in which medulla only is considered, reducing the causes of variation, may sometimes be more useful for the type of evaluation mentioned above. However, this series also has limitations, especially in the highly pigmented types. It is clear from table 3 that as medullary pigment increases cortical pigment usually increases even more, so that in considering medulla only a considerable portion of the increase in activity is missed.

The third series is more useful when there is a time factor in the data to be correlated. It has already been shown in the first paper of this series that

many genotypes have different rates of pigment production at different stages in the hair growth cycle. The usual finding is a "pigmentation lag," or low rate of production, near the tip of lightly or moderately pigmented types. This variable factor must be taken into account in collecting data where the figure obtained represents the condition at a particular moment rather than over a long period of time as, for example, the enzyme concentration at a certain stage in a growing hair follicle. BRAUCH and RUSSELL (1946a) have been very careful to measure the dopa reaction at a well-selected uniform stage, from six day old animals, in which the mid-region of the hair of the first pelage is developing. The data in table 4 are probably the best histological material avail-

TABLE 5

A comparison of pigment volume in corresponding dilute and intense genotypes. The first column of figures is the ratio of the volume of cortical and medullary pigment in dilute to intense (dd/DD) against each background, the second the ratio of medullary volumes only, and the third of the medullary volume of the mid-region.

BACKGROUND	RATIO *dd/DD* TOTAL	RATIO *dd/DD* MEDULLA	RATIO *dd/DD* MID-MEDULLA
aaBBCCPP	0.79	1.06	1.09
aaBB$c^{ch}c^{ch}$PP	1.24	1.48	1.60
aabbCCPP	0.97	1.13	1.10
aaBBCCpp	1.48	1.65	1.63
aabbCCpp	1.51	1.66	1.74
A^vaBBCCPP	1.14	1.33	1.42
A^vaBB$c^{ch}c^{ch}$PP	1.00	1.11	1.08
A^vabbCCPP	1.26	1.53	1.53
A^vaBBCCpp	1.69	1.76	1.95
A^vabbCCpp	2.36	2.56	2.59

able for comparison with their dopa results. Other workers who have timed their experiments differently could easily prepare from tables 2 and 3 of the first paper a different series of granule numbers to suit their needs.

Thus by obtaining a value proportional to the mean volume of the pigment granules and combining it with already available data on granula number, it has been possible to estimate the pigment content of each genotype tested. Data have been given for the purposes of comparison with extracts of pigment, for measurements of effects of genic substitution, and for comparison of pigment deposition at a certain stage with enzyme concentration at the same stage.

As this paper is intended solely as an addition to the body of data on variable granule attributes, the discussion should be restricted largely to a simple presentation of the differences found among the various genotypes. Further interpretation will be limited to correlation of the volume data with the condition of other granule attributes:

1. The highest members of the black-fuscous color series of genotypes

(*aaBB*) have by far the greatest volume of pigment. The highest volume in any yellow genotype is only about one-fifth that in the highest black-fuscous type, the highest brown slightly more than one-third.

2. In general within a color series the visual impression of color intensity depends more upon the total volume of pigment than upon either granule size (or shape) or number taken alone. For example, *aaBBc^{e}c^{e}DDPP* and *aaBBCCDDpp* have granules of very different size ($0.64 \pm .06\mu$, $0.38 \pm .08\mu$, table 3) and shape, the first round, the second shred-shaped. They also differ in total granule number (C+M, 230 and 381, table 3). However, the total volume of pigment is more nearly the same for the two (147 and 145, table 3), and the general appearance corresponds with the pigment volume.

3. An outstanding exception to the parallelism of pigment volume and visual impression of color is found in the dilute (Maltese) genotypes. In all cases the dilute type appears much lighter than its non-dilute counterpart. Yet in 27 of the 30 comparisons which it is possible to make (table 5, three measurements of pigment volume for each of ten corresponding pairs) between dilute and intense genotypes, the dilute has a higher pigment content than the corresponding intense type. While these data are not as accurate as could be desired, they do confirm completely the extract findings on dilute and non-dilute pairs (BRAUCH and RUSSELL 1946b). The visual effect can be attributed to the concentration of a large amount of the pigment in dilutes into the large clumps where it can have very little effect on light absorption.

SUMMARY

As a continuation of quantitative histological analysis of the action of the major coat color genes of the house mouse, this paper presents the volume of pigment, estimated in three different ways, in each of the 26 pigmented genotypes listed. The materials used were the slides prepared for the first paper of this series, and much of the data has been taken from that paper (E. S. RUSSELL 1946). The chief additional information is an estimate of the mean granule volume in each of these genotypes, based upon new measurements of the length and width of individual granules and clumps. These estimates of granule volume have been combined first with counts proportional to the total number of pigment granules in both cortex and medulla to get a series of values proportional to the total volume of pigment in each genotype, especially useful for comparison with extract data and for certain cases of quantitative evaluation of the effects of genic substitution. They have also been combined with counts proportional to the number of pigment granules in the medulla only, to get a second series of values proportional to the volume of pigment in the medulla of each genotype, which is more satisfactory for many cases of measurement of the effect of genic substitution. Still a third series of values, useful for comparison with data on enzyme concentration at a given time, was obtained by combining the mean volume estimates with counts proportional to number of medullary granules deposited during that time. Suggestions are made for the compilation of other series of pigment volumes which might be useful in other timed experiments.

The correlation of total pigment volume with other granule attributes is discussed briefly. The highest members of the black-fuscous color series are found to have by far the greatest volume of pigment. In most cases within a color series the visual appearance corresponds closely with the volume of pigment. An outstanding exception is found in types with Maltese dilution, which, although they appear much lighter than the corresponding intense types, have as high or higher total volume of pigment.

Comparable values proportional to the total volume of pigment deposited in the hair have now been obtained for 26 pigmented mouse genotypes by a single method. The results are far from perfect, but it is hoped that they are sufficiently accurate to provide a basis for interpretation of gene action.

LITERATURE CITED

BRAUCH, L. R., and W. L. RUSSELL, 1946a A study of the physiological genetics of coat color in the mouse by means of the dopa reaction. Genetics **31**: 212.

1946b Colorimetric measurement of the effects of the major coat color genes in the mouse on the quantity of yellow pigment in extracts. Genetics **31**: 212.

DUNN, L. C., and W. EINSELE, 1938 Studies of multiple allelomorphic series in the house mouse. IV. Quantitative comparisons of melanins from members of the albino series. J. Genet. **36**: 145–152.

RUSSELL, E. S., 1939 A quantitative study of genic effects on guinea pig coat colors. Genetics **24**: 332–355.

1946 A quantitative histological study of the pigment found in the coat color mutants of the house mouse. I. Variable attributes of the pigment granules. Genetics **31**: 327–346.

RUSSELL, W. L., 1939 Investigation of the physiological genetics of hair and skin color in the guinea pig by means of the dopa reaction. Genetics **24**: 645–667.

A STUDY OF THE PHYSIOLOGICAL GENETICS OF COAT COLOR IN THE MOUSE BY MEANS OF THE DOPA REACTION IN FROZEN SECTIONS OF SKIN[1]

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INTRODUCTION

AN ATTEMPT to determine the method by which genes control physiological processes seems most suitably directed toward a case in which these processes are relatively simple and lead to an end product quite local to gene action itself. Mammalian hair pigment is an intra-cellular character, produced in the melanoblasts of the hair follicle. It is present in the cells as melanin granules which can be studied histologically (WERNEKE 1916, SCHILLING 1939, DANNEEL 1936, E. S. RUSSELL 1946), or extracted and measured gravimetrically (EINSELE 1937, E. S. RUSSELL 1939), titrimetrically (E. S. RUSSELL 1939), or colorimetrically (E. S. RUSSELL 1939, HEIDENTHAL 1940, BAKER and ANDREWS 1944, BRAUCH and W. L. RUSSELL 1946b). These types of investigation have been carried out extensively on the guinea pig, mouse and rabbit, and, combined with much early work of visual grading of the total appearance of furs (WRIGHT 1927, LITTLE 1913, DUNN 1936), have led to a rather clear quantitative and qualitative picture of the end result of gene action in a great variety of genetic backgrounds.

It seems desirable, in the attempt to discover the actual mode of operation of genes, to study the biochemical process of pigment formation itself in various genetic backgrounds. Several studies of this sort have been attempted and are discussed below. The investigation reported in this paper is intended as a contribution to the field.

Autonomy of melanin containing cells

Melanoblasts of the hair follicles and basal layer of the epidermis presumably originate in the neural crest of the embryo. This has been proved for amphibians and birds by DUSHANE 1935, WILLIER 1941, EASTLICK 1939, etc. There is strong evidence for a similar origin in the mouse (RAWLES 1940, 1947).

The autonomy of pigmentation and pigmentation patterns in mammals has been proved by various transplantation experiments. Although hair formation takes place throughout adult life, the type of pigmentation seems to be fixed during fetal life; the earliest grafting experiments proving autonomy utilized dorsal tissue from 11 mm embryo donors (REED 1938a, b). Evidence for the

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local nature of gene action comes from somatic mutations, two of which involved gonadal tissue also and therefore proved the genetic nature of the change (WRIGHT and EATON 1926, DUNN 1936). Diffuse effects, as of hormones, have in rodents been reported only as exceptions.

The properties of melanin

Mammalian melanin is intra-cellular, granular and of two main types, apparently qualitatively distinct: "eumelanin" and "phaeomelanin." The latter occurs in the form of round yellow granules of rather uniform size (E. S. RUSSELL 1946) which are easily soluble in dilute alkali. Reference to "yellow melanin" is etymologically incorrect, but the long-established concept "melanin" is now being recognized to comprise a variety of probably related substances. The term eumelanin includes two types of granules which may or may not be qualitatively different: one is black or fuscous in color and of variable size and shape; the other type is brown, round and more uniform. Eumelanin is brought into dispersion with greater difficulty than phaeomelanin. Its "absorption curve" differs from that of yellow by being less steep (that is, there is relatively more absorption near the red end of the spectrum, less at the blue). Many of the more detailed interpretations which have been made from absorption spectra of melanins are probably without sufficient foundation since the curves represent dispersed rather than dissolved substances.

Since the melanins—probably complicated polymers—have never yet been conclusively analysed, the difference between phaeo- and eumelanin is a matter of speculation only. Some authors (BAKER and ANDREWS 1944, NICKERSON 1946) have assumed them to be different oxidation stages in the same reaction chain, but dopa reaction results, as will be discussed, do not support this idea. SPIEGEL-ADOLF (1937) believes that the difference in solubility alone should not be a criterion for chemical distinctness, but that the absorption spectra may speak for more than a mere difference in the state of polymerization. It seems even possible that different substrates go into the formation of the two types of pigment and this question will be discussed. Whatever the difference in formation, it must be a very labile one (E. S. RUSSELL, 1946a, proposes a "trigger mechanism" for agouti), since one and the same hair follicle can change from one process to the other (black to yellow and back to black for the sub-terminal band in agouti hairs; sootiness with increasing age in many yellows).

Conditions of melanin formation

A. Enzymes and substrates. Melanins have been formed from several phenols and possibly other organic compounds by various means. Several of the precursors spontaneously oxidise to melanin at an optimum pH and temperature. Enzymes furthering the oxidative process have been known since the end of the last century when tyrosinase was demonstrated in plants. In the animal kingdom, mealworms were found to be a good source of the enzyme. The assumption was soon made that in mammals also, tyrosin is the precursor of melanin. DURHAM (1904) and ONSLOW (1915) claimed darkening of this chromogen under the influence of an enzyme extract from rabbit and other rodent

skins—but several workers, including PUGH (1933) and DANNEEL and SCHAUMANN (1938) were unable to repeat their experiments. More consistent results were obtained using dopa (the first oxidation product of tyrosin according to RAPER 1928) as a substrate. Bloch (1927) had introduced the use of this amino acid as an indicator of pigment forming activity in melanoblasts of human skin sections and had formulated a specific "dopa oxidase" in place of the tyrosinase previously assumed. Whether there is an enzyme specific to dopa or not, dopa has subsequently been used as an indicator of at least some kind of pigment forming activity in mammalian melanoblasts, the two techniques most widely used in genetic studies being: (1) the treatment of frozen sections of skin with this chromogen (for example SCHULTZ 1925, KRÖNING 1930, and W. L. RUSSELL 1939), and (2) testing the activity of skin extracts toward dopa (for example DANNEEL and SCHAUMANN 1938, and GINSBURG 1944). The two last named studies have also yielded direct evidence for melanogenic enzymes in mammalian skin through concentration of certain active extract fractions. The work of HOGEBOOM and ADAMS (1942), although done with tumors, contributes to this evidence.

Whether dopa is oxidized by a specific dopa oxidase, as suggested by BLOCH (1927) is highly questionable. Even the "tyrosinases," obtained in several studies mostly from mushrooms and potatoes, show activity toward dopa and/or other dihydric phenols ("catecholase activity") as well as toward monohydric phenols, such as tyrosin, p-cresol, etc. ("cresolase activity"). The cresolase activity causes addition of an OH-group ortho to the one already present, the catecholase activity transforms ortho-dihydric phenols into ortho-quinones (PARKINSON and NELSON 1940). The two activities are present in definite ratios to each other and to the copper content of the extract. The cresolase, however, is more easily destroyed on standing and purification and adsorbed much more readily than the catecholase. The latter is thus demonstrated with greater ease. KEILIN and MANN (1938), who obtained only catecholase activity in purified extracts, assumed that the monophenolase activity in the original extract had been secondary and called the enzyme a polyphenol oxidase. ADAMS and NELSON (1938), however, contend that the cresolase fraction by itself shows all the properties of an enzyme and is not to be regarded as a secondary activity in the nature of a contaminant. Whatever the exact character of the enzyme active in melanin formation, the experiments of KUBOWITZ (1938), KEILIN and MANN (1938) and others have demonstrated that it is almost certainly a copper proteinate.

If one can draw conclusions as to the mammalian enzyme (which is dangerous at best since "tyrosinases" differ in their substrate affinities even among different mushrooms), it might be suggested that the failure to demonstrate conclusively enzyme activity toward monohydric phenols (i.e. tyrosin) is due to the greater instability of the "cresolase fraction" of the enzyme as compared with the "catecholase fraction" (acting consistently on dopa). Some evidence that two such activities actually exist in mammals comes from the work of HOGEBOOM and ADAMS (1942). They were able to separate a tyrosinase fraction from a dopa-oxidase fraction in melanoma extracts. The latter was not entirely specific to dopa, since it acted, though more slowly, on catechol as well.

In human skin, according to one view (ROTHMAN 1942a, b) the enzymatically controlled part of the oxidation to melanin begins with dopa. The natural substrate, however, is tyrosin which is converted to dopa by the action of ultraviolet light in the presence of non-specific catalysts, such as ferrous salts or ascorbic acid. "Photosynthetic melanins," formed from several aromatic amino acids by ultraviolet light without the aid of enzymes, have been obtained by SPIEGEL-ADOLF (1937).

B. Other possible factors. Two extract studies have produced evidence for the presence in skin of an inhibitor (or inhibitors?) to the dopa reaction. DANNEEL and SCHAUMANN (1938), working on rabbits, isolated such a substance in the albumin fraction. GINSBURG (1944) deduced the presence of an inhibitor, not affected by color genes, in guinea pig skin extracts and located it in the epidermis. Such studies with enzyme extracts make the use of adequate controls imperative since dopa spontaneously oxidizes to melanin with such ease that a mere darkening of skin extracts containing the chromogen does not prove the presence of an enzyme. That the inhibitors are probably introduced from elsewhere in the skin through the necessary crudeness of the extraction method and do not normally control dopa reaction (and natural pigmentation?) differences is indicated not only by GINSBURG'S work but by the results of the frozen section method. The appearance of dopa bathed sections makes it very apparent that an enzymatic reaction in the follicles is not obscured by inhibition. Though no controls analogous to those used in extract work can be employed, the tissue surrounding the follicles serves as control area which is almost white whereas the follicles contain varying amounts of dark dopa melanin.

Another factor that has been suggested as possibly controlling the oxidation of the chromogen—other than the balanced system of enzyme(s) and inhibitor(s)—is the oxidation-reduction potential of the pigment forming cell. FIGGE (1940) finds tyrosinase active in only a relatively small range of potentials. Hallachrome, one of the intermediate products in melanin formation from tyrosin, sets up a reversible redox system which establishes the potential near optimum for the continuation of the reaction. In salamanders there seems to be an initial difference in the potentials of melanophores and connective tissue cells, such that one type will allow the oxidation of tyrosin whereas the other will not. The same agent shifts the potentials of both types of cell in the same direction, bringing the first type out of and the second type into the optimum range. A somewhat similar situation may explain the difference in dopa reaction found between basal layer and hair follicle cells in the guinea pig by W. L. RUSSELL (1939). In that case, a gene substitution such as *pp/P* may cause a shift in one direction, that of *ff/F* in the opposite direction. Tests of such a situation have, however, so far been negative.

Purpose of present study

It may be possible to relate many of the biochemical processes recently discovered to the action of the several series of coat and skin color genes. Since

dopa has been found by W. L. RUSSELL to provide such a sensitive indicator of some phase of gene-controlled differences in the guinea pig, we have used it in a similar study on the mouse.

A point of considerable biochemical interest arose from the work on the guinea pig. W. L. RUSSELL (1939) by the frozen section method (later confirmed by the extract study of GINSBURG 1944) found that the reaction in the hairbulbs measured not, as had long been supposed, the sepia pigment producing capacity of the cells, but the yellow-producing capacity as expressed in *ee* genotypes.[3] In the yellow (*ee*) genotypes, the grades of reaction paralleled the degrees of pigmentation present. In other genotypes tested (including black, brown and pale sepia animals) the reaction corresponded not with the pigmentation present in the cells but with that in the corresponding *ee* genotypes. Thus, a gene substitution such as *pp* for *P*-, which reduces the intensity of sepias but has no effect on yellows, also does not affect the dopa reaction, whether it is carried out in a *pp* yellow or a *pp* sepia. The primary interest in the present study was to determine whether or not a similar parallelism held in another mammal. The mouse is favorable material for such a comparison not only because of the abundance of known color genes but also because it differs from the guinea pig in the location and dominance relations of the yellow gene. A^y in the mouse is dominant (homozygous lethal), in contrast to *e* in the guinea pig; it is, moreover, an allele of agouti, light-bellied agouti, non-agouti and black-and-tan, while the *e* gene of the guinea pig is at a separate locus.

[3] With one possible exception, a full coat of yellow in the guinea pig is produced only in the presence of *ee*. The exception is *E-ffpp*. WRIGHT classifies the pigment in this genotype not as pale sepia (as would be expected from other *E-pp* combinations) but as yellow and of a lower grade than *eeffpp*. If the pigment in *E-ffpp* actually is phaeomelanin, then, in the combination *ffpp*, *E* must be regarded as a diluter of yellow. WRIGHT explains this by assuming two things: (1) That in all sepia (*E*) combinations there is present an underlying yellow (or uncolored precursor of yellow) which, however, is less than in the corresponding *ee*-combinations because of a competition by the sepia producing process for some common substance. (2) That the gene *F* has a double effect: (a) the contribution of an essential component for yellow pigment formation (*f* furnishes this in smaller amounts), and (b) a feeble substitution for *P* in the sepia forming process, such that in the absence of both *F* and *P* (that is in *ffpp*) no sepia pigment is produced. On these assumptions, the pigment in *E-ffpp* animals is regarded as the underlying yellow, and the fact that it is less in amount than in the corresponding *eeffpp* combination is explained as a result of the competition by the sepia-producing process which, because of the presence of *ff* and *pp*, never reaches its final step.

The dopa results show little or no difference between the two genotypes, *E-ffpp* and *eeffpp* (only two cases in W. L. RUSSELL's publication, but confirmed in unpublished work by APPEL, 3 more cases, and BRAUCH, 9 more cases). If the pigment in *E-ffpp* is really yellow, as WRIGHT believes, then it must be said that the dopa results do not follow yellow in this comparison (or the reaction would be of a lower grade in this genotype than in the more intense *eeffpp* yellow). Even in this case, however, the dopa reaction does, as stated as a general conclusion in the text, still parallel the *yellow-producing capacity of the corresponding ee genotype*. This result yields a simpler physiological genetic interpretation than if the reaction in *E-ffpp* had corresponded with the reduced yellow pigment present, for the dopa reactions in other *E*-combinations are certainly not less than in the corresponding *ee*-combinations. Thus, consistently throughout all *E*-genotypes, the dopa reaction is not affected by the competition that WRIGHT postulates as taking place between the eumelanic and phaeomelanic processes.

MATERIALS

Source of animals

Seventy-three genotypes were tested, using one to six animals of each. Seventy of these (including six W^v combinations) came from color stocks maintained by W. L. RUSSELL and E. S. RUSSELL. Three miscellaneous color mutations (leaden, misty and piebald), present only in single combinations, were also tested in Jackson Laboratory stocks maintained by DRS. CLOUDMAN, WOOLLEY and SNELL respectively.

All yellow animals were A^va and came from matings of A^va by *aa*. This made it possible to obtain a more valid comparison of corresponding sepia and yellow types by using *aa* and A^va littermates. We tried, however, to avoid using more than one such pair from the same litter or even, if possible, from the same sibship, attempting thereby to obtain a better average value for each stock and a rough measure of the variability within stocks.

The regular color stocks had been maintained for from one to several years. Some had become slightly inbred, although this was prevented, when practicable, for the sake of maintaining vigor and to avoid fixing modifiers too differently in different stocks. Albino series heterozygotes used were usually the first generation animals from crosses between stocks. In tables and figures and occasionally in the text, each genotype is indicated only by those genes in which it differs from the formula *BBCCDDPP*, also referred to as "type." The *aa* background is usually implied in this term, unless "A^va" is added.

All regular color stock as well as W^v animals used were as nearly as possible 6–7 days old since it was determined that at this age the majority of the follicles are producing that part of the hair which corresponds to E. S. RUSSELL'S 20–40th fields and which was selected by her as a convenient region for comparison of genotypes (E. S. RUSSELL 1946). In some types (particularly $c^{ch}c^{ch}pp$ and $c^{ch}c^{e}pp$) it was very hard to distinguish the A^va and *aa* animals in a litter at that age. Animals in those litters were therefore not sacrificed but kept alive (only a small piece of skin was removed for sectioning) to check on their genotype when the coat was more fully grown.

Leaden, misty and piebald animals used were slightly older than the standard age. Litters did not segregate for A^va and *aa*.

Genetic Background

The study of the interaction effects of the major color genes in the mouse has not been as extensive or quantitative as in the guinea pig (WRIGHT 1941; review, 1942). There is, for instance, nothing available comparable to the visual grading by WRIGHT (1927), although DUNN (1936) has given a rough numerical description to define the order of visible effects of the albino series of genes on yellow, black and brown backgrounds. Some pigment extraction work has been done by DUNN and EINSELE (1938) for black and brown light-bellied agouti mice, in which genotypes they measured the size of granules extracted and also made colorimetric comparisons. However, none of these determinations was more than an estimate and the genotypes investigated were

few. An extensive histological study has recently been made by E. S. RUSSELL (1946) and we are basing some detailed aspects of our discussion on her results. Data more recently obtained by us from a colorimetric study of pigment extracts from a variety of yellow genotypes (to be published, abstract BRAUCH and RUSSELL 1946b), will also be referred to in the discussion.

The more thoroughly investigated color genes of the mouse form five allelic series, combinations of which have been used in this study.

(1) Agouti series, A^v, A^w, A, a^t, a. This determines the fundamental kind of melanin to be formed as well as its distribution on the animal and in the individual hair. Only A^v and a were used in this experiment. *aa* hairs contain only eumelanin, A^va hairs normally only phaeomelanin.

(2) Black-brown: *B*, *b*. After the primary differentiation into phaeo- and eumelanin, brought about by the agouti series, the genes at the *b*-locus seem to control a secondary (probably qualitative) differentiation of the eumelanin into black or brown. In sepias (that is, *aa* animals in our case), *bb* changes granule color to brown as well as preventing enlargement or elongation of granules and reducing the number slightly in some backgrounds. It has little if any effect on yellows.

(3) Albino series: C, c^{ch}, c^e, c^a. This causes a gradual reduction from a maximum quantity of pigment to no pigment at all, the steps in the reduction varying according to the genetic background.

(4) Pink-eyed dilution, *pp*, apart from eliminating pigment from the eye, has its main effect in very greatly reducing eumelanin in the hair while causing little if any visible change in yellows. Microscopically, however, hairs of A^vapp as well as *aapp* combinations—though to a much smaller extent—give evidence of pigmentation lag (that is, relatively fewer granules in the tip of the hair) as well as diminution of cortical pigment.

(5) Maltese dilution, *dd*, causes a dulling and apparent dilution of the coats of both sepia and yellow genotypes. E. S. RUSSELL (1946) finds a queer type of clumping in the hair of dilutes, as well as a diminution of cortical pigment, and a large range of granule size in the case of sepias. In the course of the present study, it seemed as though the hair bulbs of "dilute" types had as much or more pigment than those of the corresponding *DD*. This observation was later confirmed by extracting the skin and fur pigment of yellow genotypes (BRAUCH and W. L. RUSSELL 1946b) and for the hair itself of both yellows and sepias by E. S. RUSSELL (1948). The appearance of the granule clumps within the follicle is almost as though the pigment were somehow held back and prevented from reaching the hair except in occasional bursts. The effect of the dopa reaction in "dilute" animals is also peculiar and will be discussed below.

Partly in response to the need shown by this experiment for some unified quantitative statement of natural pigment in various mouse genotypes, E. S. RUSSELL has attempted histological estimates of total pigment volumes. These are based on the mean granule volume for each genotype (E. S. RUSSELL 1948) and granule counts proportional to the total number per hair or hair region of that type (E. S. RUSSELL 1946). No albino series heterozygotes were studied by her. Since, in timing our experiment, we have attempted to get a measure of

TABLE 1

Comparison of histologically estimated volumes of natural pigment (from E. S. RUSSELL, *1946) with dopa reaction grades in various aa and A^va genotypes.*

	Table 1a				Table 1b			
	aa				*A^va*			
	MID REGION COUNT (C AND M)*	MEAN GRANULE VOLUME	TOTAL VOL. AS % OF "TYPE"	DOPA GRADE AS % OF GRADE IN "TYPE"	MID REGION COUNT (C AND M)*	MEAN GRANULE VOLUME	TOTAL VOL. AS % OF "TYPE"	DOPA GRADE AS % OF GRADE IN "TYPE"
Type	298	1.53	100	(100)**	132	0.63	100	100
$c^{ch}c^{ch}$	301	0.79	52.2	(50)	89	0.55	58.9	69.9
c^ac^a	118	0.64	16.6	(25)	5	?	?	3.8
c^ac^a	0	—	0.0	0.0				
bb	295	0.63	40.8	101.5	119	0.61	87.3	102.5
$bbc^{ch}c^{ch}$	273	0.62	37.1	(50)	72	0.51	44.2	56.1
$bbc^a c^a$	137	0.62	18.6	(37.5)	1	?	?	13.4
pp	188	0.38	15.7	100.8	121	0.66	96.0	103.5
$c^{ch}c^{ch}$ *pp*	114	0.37	9.3	34.5	62	0.41	30.6	47.7
$c^a c^a$ *pp*	0	—	0.0	0.0				
$c^a c^a$ *pp*	0	—	0.0	0.0				
dd	217	2.25	107.1	(100)	121	0.96	139.7	113.6
$c^{ch}c^{ch}$*dd*	262	1.51	86.8	(50)	47	1.12	63.3	72.2
bb pp	151	0.33	10.9	98.0	113	0.45	61.2	100.3
bb dd	170	1.23	45.9	(100)	93	1.16	129.7	110.1
ddpp	134	0.86	25.3	104.0	124	1.26	187.9	103.8
bb ddpp	122	0.71	19.0	104.5	120	1.10	158.7	102.3

* C = cortex, M = medulla.

** Percentages in parentheses are derived from estimated grades (see text).

pigment forming activity in the characteristic "midregion" of the hairs (see "Source of animals" above) we have used the sum of the cortex and medulla counts for the 20th, 30th and 40th fields (see first columns of tables 1a and 1b, computed from E. S. RUSSELL 1946) in the product with the granule volumes (second columns) to derive some expression for the relative quantities of natural pigment in that region. Eumelanin and phaeomelanin are then given as percentages of *aaBBCCDDPP* and *A^vaBBCCDDPP* respectively. This is admittedly not a perfect statement of relative pigment volumes but should be sufficient to show an order or grouping of genotypes on which comparisons with dopa reactions can be based.

A number of color mutations which, so far, have not received as much study as the five loci discussed above were tested in a few backgrounds:

(1) Dominant spotting, *W*, W^v, *w*. Combinations of two of this series of three alleles were studied, namely W^vW^v, W^vw and *ww* in segregating litters. Some or all of these segregants were available in the backgrounds *aapp*, *aabb*, A^va and *aa*, and came from stocks being inbred by E. S. RUSSELL. W^v, apart from its effect on the blood, fertility, etc., is a gene for both coat color and pattern, causing a slight dilution as well as white spotting in W^vw heterozygotes. W^vW^v animals are white.

(2) Leaden, *ln*, available on an *aabb* background, phenotypically resembles Maltese dilution, but *aabblnln* is said to be somewhat lighter than *aabbdd* and microscopically shows more pigment clumping (GRÜNEBERG, 1943, p. 40).

(3) Misty, *m*, was present with *aabb* and *aabbdd*. This mutation dilutes fur color slightly, but—in gross appearance—not as much as *lnln* or *dd* (WOOLLEY 1941). Its effect on yellow has not yet been tested.

(4) Piebald, *s*, produces white spotting of varying extent, white and colored areas being generally clearly defined with sharp borders. This gene was present on *aa*.

METHOD

As mentioned above, dopa has been used as artificial chromogen in extract experiments as well as in frozen sections of skin. Points can be cited in favor of both these procedures, but the following advantages of the frozen section method made us adopt it for this work:

(1) The extracting method is not fine enough to separate merely the enzyme (?) from the skin. Other substances included in the active fraction may complicate the results. (a) Polymorphonuclear leucocytes, for instance, have been found to give the reaction (BLOCH 1927). (b) GINSBURG (1944) found in the epidermis an "inhibitor" to enzyme activity. It appears, however, that the epidermis cannot be efficiently removed and fine gradations of reaction are not obtained with accuracy. It is conceivable that the reaction is upset by a number of unfavorable conditions or substances called into play unless the disturbance of the natural system is minimized.

(2) The frozen section method permits a histological location of activity. Thus W. L. RUSSELL (1939) found different reactions in the hair bulbs and the basal layer of the epidermis and we found a peculiar effect of the *d* gene which will be discussed below.

Procedure

An attempt was made by W. L. RUSSELL (1939) to find the most favorable conditions for demonstrating differences in the reactions of different genotypes in the guinea pig. The techniques of previous workers were examined and controversial points tested experimentally. The method finally adopted has, therefore, been followed closely in the present work, making comparison between the mouse and the guinea pig as legitimate as possible. The mention of any minor departures is included in the following summary of the procedure.

(1) Fixation. A piece of skin, removed mid-dorsally from the lumbar region,

was fixed in five percent formalin for about two hours. It was found that even in one of the weakest reacting types, $c^e c^e$, immersion in formalin for as long as two days had no weakening effect on the dopa melanin formed, thereby verifying the guinea pig results. The period of fixation was usually two hours and never exceeded four.

(2) Sectioning. The skin was rinsed in distilled water and cut into 30 micron sections on the freezing microtome (the chemicals used in the technique of paraffin embedding destroy the reaction) being careful to cut at right angles to the surface of the skin and in the plane of the hairshafts. Thirty to forty sections were immersed in the experimental solution and an approximately equal number in the control solution.

(3) Solutions.

EXPERIMENTAL	CONTROL
5 cc 1 g/1000 cc dopa	5 cc distilled water
5 cc phosphate buffer	5 cc phosphate buffer

The dopa concentration was thus 1 g/2000 cc, which is equal to that used by W. L. RUSSELL (1939) although the amounts of solution used were smaller to conserve materials. The buffer consisted of 2M/15 solutions of secondary sodium phosphate and primary potassium phosphate in the proportion of 4:1 by volume, giving a pH of 7.4. The water used for all solutions was ordinary laboratory distilled water which was found to be sufficiently pure (W. L. RUSSELL, 1939, had used double distilled water). The dopa solution was always made up less than two hours before use and not mixed with the buffer until just before the sections were immersed.

(4) Incubation. The experimental temperature was 39.5–40°C. This and the pH of 7.4 are not exactly optimum conditions for the formation of dopa melanin but presumably approximate those at which the reaction takes place in the body of the animal. Sections were incubated for a total period of three hours. At the end of the first hour, the 15 best sections in each dish were transferred to fresh replacing solutions. Due to a shortage of dopa, W. L. RUSSELL's (1939) procedure of changing the solutions twice could not be followed, but it was believed that one replacement would accomplish the purposes of: (1) removing traces of formalin which might inhibit the reaction and (2) preventing excessive dopa melanin to be formed by auto-oxidation. The replacing solutions were identical with the original ones, dopa and buffer again being mixed just before use.

At the end of the incubation period, the sections were removed, rinsed in distilled water, passed through the alcohols and xylol, and the five to six best ones mounted in balsam.

Measurement of results

The dopa reaction leads to amorphous shreds of *sepia* pigment in the hair bulb and, sometimes, for a short distance up the shaft. It is unknown whether granules similar to the granules of natural pigment are formed and exactly

where they are located, since the sections are too thick to use magnifications higher than about 400× profitably. Some reaction also occasionally occurs in dendritic cells in the skin, but these cells are so much rarer than in the guinea pig and the reaction in them so erratic that no attempt was made to grade the melanin in that location.

Some preliminary work was done arranging slides in an approximate order of increasing reaction and it was found that, as in the guinea pig, visual discrimination allowed six degrees of intensity. Follicles were then chosen as standards to represent these steps which are recorded as grades 0–5. Although the grades are thus the same as in the guinea pig, there is, of course, no foundation for absolute comparisons. Any grading of this sort establishes merely an order of effect. It must be pointed out that the grades probably do not represent an arithmetical progression but more or less uniform percentage steps in the actual quantities of dopa melanin formed, since small changes in intensity are more easily perceived when the total intensity level is low (for example, the increment in dopa melanin between grades 1 and 2 is presumably much less than between grades 4 and 5). Scale transformation was not attempted since order of effect rather than absolute quantities was all that was needed for the interpretations desired in this paper.

The grade for a particular animal represents the average for the hair bulbs in the sections. We had originally planned to grade only follicles cut through the papilla, but, in order to increase the number on which the average was based, decided to include all follicles except those cut very far from the center. This decision was reached after we had made a few preliminary comparisons between the two ways of grading and had found no great difference. Since, at the age used, only active, living follicles are present, no special policy concerning the grading of club hair bulbs had to be adopted.

Comparison of experimental with control sections led to a division of the material into two groups. In the smaller of the two, the dopa melanin is in some manner not easily distinguishable from the natural pigment present and exact grading is impossible. This group (seventeen genotypes in all) contains all eumelanic types (that is, *aa*) except (1) those having very little pigment and fine granules, namely pink-eyed sepias, and (2) a few browns in which the reaction is strong enough to stand out clearly from a background qualitatively somewhat different. The closest approximation to a grade which could be obtained in the 17 genotypes of this group was an estimate of the range of the reaction in the various follicles of a slide. The results for 16 of the types are given in table 3, the seventeenth ($aaW^{v}w$) is included in table 5.

The experimental sections of the larger group (53 genotypes) were graded directly against the standard bulbs. The results are shown in tables 2 and 5, which may be seen to include all yellows as well as those exceptional sepias mentioned above where the reaction, though dark like eumelanin, is still clearly distinguishable from the natural pigment. Each row in the tables represents the reaction in a single animal and shows the number of follicles falling within each grade as well as the mean grade for the animal.

TABLE 2

Distribution and means of dopa reaction grades in individual animals.

(a)

	$A^{y}a$							$A^{y}app$							pp						
	0	1	2	3	4	5	MEAN	0	1	2	3	4	5	MEAN	0	1	2	3	4	5	MEAN
CC	—	—	—	23	271	7	3.95	—	—	—	17	163	19	4.01	—	—	—	44	134	19	3.87
	—	—	—	31	279	19	3.96	—	—	—	2	312	49	4.13	—	—	—	30	164	45	4.06
	—	—	—	8	150	2	3.96	—	—	—	18	200	64	4.16	—	—	2	32	217	97	4.15
Cc^{ch}	—	—	—	39	176	5	3.85	—	—	—	47	161	5	3.80	—	—	15	213	179	1	3.41
	—	—	—	16	172	25	4.04	—	—	—	53	221	18	3.88	—	—	1	109	105	4	3.51
								—	1	—	39	246	6	3.88	—	—	2	120	259	3	3.69
															—	—	—	94	254	11	3.77
															—	—	—	68	128	18	3.77
Cc^{a}	—	—	—	46	223	6	3.85	—	—	—	83	143	3	3.65	—	—	2	88	112	1	3.55
	—	—	—	25	139	23	3.99	—	—	1	48	158	4	3.78	—	—	11	97	168	21	3.67
Cc^{a}	—	—	—	51	155	1	3.76	—	—	21	92	128	1	3.45	—	—	30	131	66	—	3.16
	—	—	—	21	163	23	4.01	—	—	2	86	177	2	3.76	—	—	24	121	112	—	3.34
$c^{ch}c^{ch}$	—	10	53	107	18	—	2.71	13	32	62	35	—	—	1.84	12	193	11	—	—	—	1.00
	—	6	21	60	7	—	2.72	7	39	150	24	—	—	1.87	8	153	52	—	—	—	1.21
	—	8	25	259	3	—	2.87	1	20	81	11	—	—	1.90	26	118	99	17	—	—	1.41
								17	35	163	44	—	—	1.90	8	42	109	—	—	—	1.64
								19	30	129	56	—	—	1.95	17	71	144	16	—	—	1.64
$c^{ch}c^{a}$	18	71	90	1	—	—	1.41	45	89	120	6	—	—	1.33	29	57	35	—	—	—	1.05
	23	113	147	—	—	—	1.44	26	82	116	—	—	—	1.40	6	36	27	—	—	—	1.30
								64	72	194	1	—	—	1.40	25	94	84	4	—	—	1.32
								15	96	183	—	—	—	1.56	9	72	55	—	—	—	1.34
															4	87	94	2	—	—	1.50
															7	62	43	14	—	—	1.51
$c^{ch}c^{a}$	43	139	66	—	—	—	1.09	70	70	—	—	—	—	0.50	35	43	—	—	—	—	0.55
	14	100	47	—	—	—	1.20								39	53	—	—	—	—	0.57
	7	77	54	—	—	—	1.34								90	149	—	—	—	—	0.62
															63	110	—	—	—	—	0.64
															82	171	—	—	—	—	0.68
$c^{a}c^{a}$	100	15	—	—	—	—	0.13								34	—	—	—	—	—	0.00
	129	20	—	—	—	—	0.13								161	—	—	—	—	—	0.00
	148	22	—	—	—	—	0.14														
	174	46	—	—	—	—	0.21														
$c^{a}c^{a}$	215	3	—	—	—	—	0.01								178	—	—	—	—	—	0.00
	133	61	—	—	—	—	0.31								140	—	—	—	—	—	0.00
$c^{a}c^{a}$															98	—	—	—	—	—	0.00
															143	—	—	—	—	—	0.00

(f)

	Type						
	0	1	2	3	4	5	MEAN
$c^{a}c^{a}$	325	37	—	—	—	—	0.10
$c^{a}c^{a}$	268	—	—	—	—	—	0.00
	164	—	—	—	—	—	0.00

(b)

	$A^v abb$							bb							$A^v add$						
	0	1	2	3	4	5	MEAN	0	1	2	3	4	5	MEAN	0	1	2	3	4	5	MEAN
CC	—	—	—	28	186	10	3.92	—	—	3	46	225	14	3.87	—	—	—	—	187	113	4.38
	—	—	—	10	141	32	4.12	—	—	—	34	264	7	3.91	—	—	—	16	101	137	4.48
	—	—	—	16	304	76	4.15	—	—	—	18	534	116	4.15	—	—	—	—	74	88	4.54
								—	—	—	—	261	121	4.32	—	—	—	9	71	146	4.61
Cc^{ch}	—	—	—	33	148	22	3.95	—	—	—	42	200	15	3.90	—	—	5	33	147	10	3.61
								—	—	—	13	168	17	4.02	—	—	2	26	108	5	3.82
Cc^{a}	1	1	22	82	168	2	3.53	—	—	—	36	232	11	3.91							
	—	—	4	72	156	1	3.66	—	—	—	33	236	16	3.94							
$c^{ch}c^{ch}$	11	43	142	39	—	—	1.89								9	22	52	121	19	—	2.51
	—	27	213	88	6	—	2.22								9	18	30	172	43	—	2.82
	1	22	86	183	—	—	2.54								—	—	12	81	36	5	3.25
$c^{ch}c^{a}$	38	79	87	—	—	—	1.24														
	26	78	87	2	—	—	1.34														
$c^{ch}c^{a}$	30	65	24	—	—	—	0.95														
$c^{a}c^{a}$	105	46	2	—	—	—	0.33														
	57	53	1	—	—	—	0.50														
	101	110	—	—	—	—	0.52														
	75	115	5	—	—	—	0.64														
	60	113	—	—	—	—	0.65														

(c)

	$A^v addpp$							$ddpp$							$A^v abbdd$						
	0	1	2	3	4	5	MEAN	0	1	2	3	4	5	MEAN	0	1	2	3	4	5	MEAN
CC	—	—	2	17	148	32	4.06	—	—	1	47	171	38	3.96	—	—	—	16	158	56	4.17
	—	—	—	9	160	29	4.10	—	—	—	26	165	85	4.21	—	—	—	—	131	42	4.24
	—	—	1	11	123	45	4.18	—	—	—	12	160	98	4.32	—	—	—	4	157	146	4.46
															—	—	—	1	123	172	4.58

(d)

	$A^v abbpp$							$bbpp$						
	0	1	2	3	4	5	MEAN	0	1	2	3	4	5	MEAN
CC	—	—	5	64	238	11	3.80	—	—	5	58	230	8	3.79
	—	—	—	36	320	17	3.95	—	—	1	33	231	18	3.94
	—	—	—	14	316	17	4.01	—	—	—	39	501	23	3.97
	—	—	—	23	147	47	4.11	—	—	—	46	368	36	3.98

(e)

	$A^v abbddpp$							$bbddpp$						
	0	1	2	3	4	5	MEAN	0	1	2	3	4	5	MEAN
CC	6	2	1	13	172	11	3.83	—	—	—	32	162	38	4.03
	—	—	—	6	200	11	4.02	—	—	—	15	260	62	4.10
	—	—	1	24	382	43	4.04	—	—	—	8	117	52	4.25
	—	1	—	13	135	92	4.31	—	—	—	14	282	182	4.35

TABLE 3

Estimated dopa reaction grades in genotypes in which the amount of natural pigment prevents accurate grading of the reaction.

	Type	*dd*	*bb*	*bbdd*
CC	277 bulbs (3–5) 328 bulbs (3–5)	206 bulbs (3–5) 253 bulbs (3–5) 365 bulbs (3–5) 131 bulbs (3–5)		396 bulbs (3–5) 418 bulbs (3–5) 367 bulbs (3–5) 214 bulbs (3–5)
Cc^{ch}	246 bulbs (2–5) 184 bulbs (3–5)	180 bulbs (3–5)		
Cc^{a}	288 bulbs (2–5) 240 bulbs (3–5)			
Cc^{a}	200 bulbs (2–4) 228 bulbs (2–5)			
$c^{ch}c^{ch}$	258 bulbs (1–3) 305 bulbs (1–3) 228 bulbs (1–3)	219 bulbs (0–3) 227 bulbs (0–3) 248 bulbs (2–4)	345 bulbs (1–3) 315 bulbs (1–3) 198 bulbs (1–3)	
$c^{ch}c^{a}$	217 bulbs (0–2) 162 bulbs (0–3)		215 bulbs (0–3) 270 bulbs (0–3)	
$c^{ch}c^{a}$	311 bulbs (0–2) 285 bulbs (0–2) 243 bulbs (0–2) 119 bulbs (0–2)		216 bulbs (0–2)	
$c^{a}c^{a}$	264 bulbs (0–1) 247 bulbs (0–1) 247 bulbs (0–2) 310 bulbs (0–2)		245 bulbs (0–1) 187 bulbs (0–2) 149 bulbs (0–2) 209 bulbs (0–2) 343 bulbs (0–3)	

RESULTS AND DISCUSSION

Table 4, prepared from tables 2 and 3, summarizes dopa reaction results for combinations of the five better known allelic series. The means given for genotypes in which there was direct grading are determined from the means of all the animals of that type tested. In the other genotypes, where the grade is an estimate, the range given in table 14 is for all the follicles of the various animals of the particular genetic constitution. The standard errors of the means in table 4 are calculated from generalized variances[4] obtained for two groups of genotypes (those with means above 0 but less than 2; and those with averages above 2) from the following formula:

[4] This statistical treatment was suggested by DR. S. WRIGHT.

$$\sigma^2 = \frac{\sum_1^k \left(\sum_1^n v^2 - \bar{v} \sum v \right)}{\sum_1^k (n - 1)}$$

where n = the number in a particular genotype
$v = \sum_1^n v/n$, the genotype mean
k = the number of genotypes in a group

In the former group of 14 genotypes, the variance was 0.0203, in the latter group of 30 it was 0.0259.

On first gross examination of the data it becomes apparent that the reaction is approximately equal in corresponding *aa* and $A^v a$ genotypes and that only the *C*-series acts markedly on its intensity. Neither the substitution of *bb* nor of *pp* seem to have a great effect. (See below for finer tests. Since our abstract was submitted, 1946a, more data have accumulated which have altered the results originally reported in one or two details.) It may be noted that this failure to be modified by *bb* and *pp* holds roughly for natural phaeomelanin, while the same gene substitutions affect eumelanin quite radically. The parallelism of the dopa reaction in *aa* as well as $A^v a$ types with yellow pigment thus becomes apparent even in the crudest analysis of the data and is sustained in all the steps of a more detailed examination.

Table 1 and figure 1 illustrate the parallelism more clearly. The third columns in each of the two parts of table 1 contain (as already described) estimates of relative pigment volumes in the hair midregions of various genotypes (based on E. S. RUSSELL's data). Dopa grades in sepias and yellows, converted into percentages of the grades in *aaBBCCDDPP* and $A^v aBBCCDDPP$ respectively, are given in the next column of the same table. Figure 1 has been constructed from these two sets of data contained in table 1. Although we have more direct measurements of pigment volumes from the extraction of phaeomelanins in a variety of $A^v a$ types (to be published), we have based the diagram on E. S. RUSSELL's estimates in order to have equivalent data for sepias and yellows. It may be pointed out again that the reaction, regardless of the genotype in which it occurs, produces pigment of similar appearance to eumelanin.

The effects of various gene substitutions on the reaction lend themselves to statistical analysis by the method of paired comparisons, since most of the substitutions studied were available on a number of genetic backgrounds. In the test, the types paired differ only in the alleles under consideration, for example, in comparing *BB* with *bb*, $A^v aBB$ is paired with $A^v abb$, $A^v aCc^{ch}BB$ with $A^v aCc^{ch}bb$, $A^v aCc^e BB$ with $A^v aCc^e bb$, etc. for 12 comparisons in all. By this method, the substitution of *bb* for *BB* is found to have no significant effect on the dopa reaction (P = 0.15).

Surprising results were obtained with *dd* combinations. As far as can be judged, they give a stronger reaction than the corresponding *DD* types in the four comparisons where only estimates can be made. In the eight comparisons where exact grading is possible, the difference is not significant (P = 0.11), but

TABLE 4

Dopa reaction grades in each genotype (from mean of means in table 2 and total estimates in table 3). Standard errors are derived from generalized variances calculated for two groups of means (see text).

TYPE	*dd*		*pp*		*ddpp*		*bb*		*bbdd*		*bbpp*		*bbddpp*	
	A^va	*aa*	A^va	*aa*	A^va	*aa*	A^va	*aa*	A^va	*aa*	A^va	*aa*	A^va	*aa*
CC	3.96 (3–5) ± .09	4.50 (3–5) ± .08	4.10 ± .09	4.03 ± .09	4.11 ± .09	4.16 ± .09	4.06 ± .09	4.06 ± .08	4.36 (3–5) ± .08		3.97 ± .08	3.92 ± .08	4.05 ± .08	4.18 ± .08
Cc^{ch}	3.95 (2–5) ± .11	3.72 (3–5) ± .11	3.85 ± .09	3.63 ± .07			3.95 ± .16	3.96 ± .11						
Cc^e	3.92 (2–5) ± .11		3.72 ± .11	3.61 ± .11			3.60 ± .11	3.93 ± .11						
Cc^a	3.89 (2–5) ± .11		3.56 ± .11	3.25 ± .11										
$c^{ch}c^{ch}$	2.77 (1–3) ± .09	2.86 (0–4) ± .09	1.89 ± .06	1.38 ± .06			2.22 (1–3) ± .09							
$c^{ch}c^e$	1.43 (0–3) ± .10		1.42 ± .07	1.34 ± .06			1.29 (0–3) ± .10							
$c^{ch}c^a$	1.21 (0–2) ± .08		0.50 ± .14	0.61 ± .06			0.95 (0–2) ± .14							
c^ec^e	0.15 (0–2) ± .07			0.00			0.53 (0–3) ± .06							
c^ec^a	0.16 ± .10	0.10 ± .14		0.00										
c^ac^a		0.00		0.00										

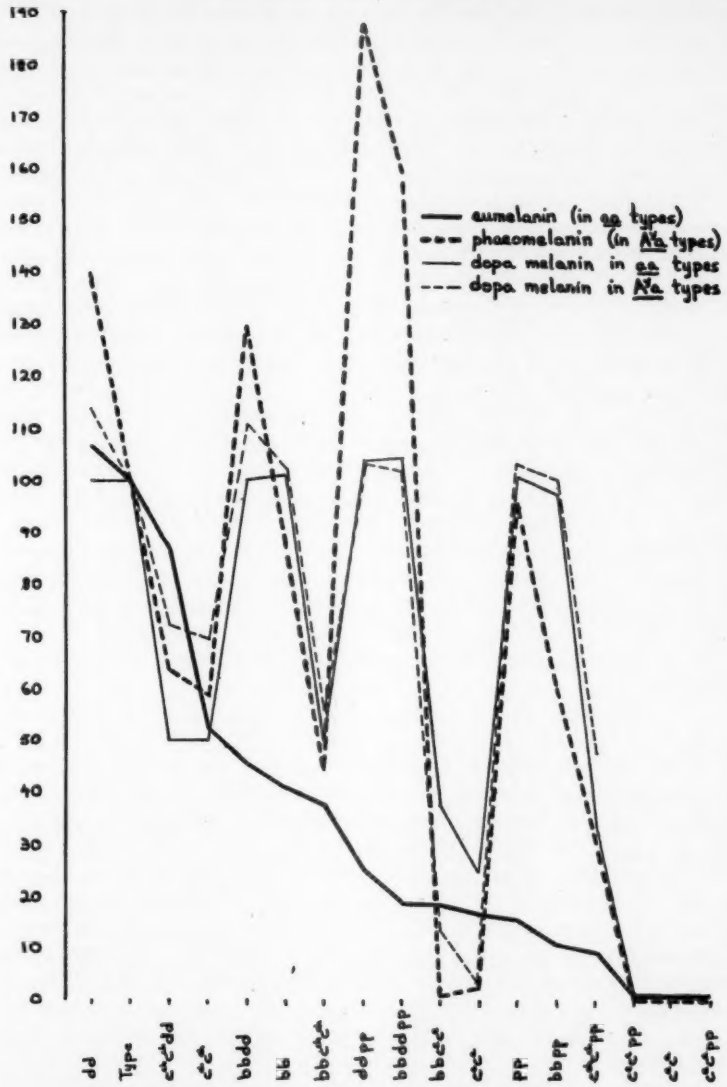

FIGURE 1.—Comparison between natural pigment and dopa reaction in various A^va and *aa* genotypes. Genotypes are arranged in order of decreasing pigmentation in *aa* types. All values are from table 1 (see text).

the *dd* means are higher than the *DD* in seven of the eight cases. Since the *d* gene had been considered a diluter of yellow as well as sepia pigment, a decreased dopa reaction in *dd* animals was expected. The observed increase seemed to present the first case of failure of the reaction to parallel effects on yellow. However, colorimetric measurement of yellow pigment in extracts (BRAUCH and RUSSELL 1946b) yielded values for "dilute" genotypes higher than for corresponding non-dilutes. E. S. RUSSELL'S recently calculated total granule volumes confirm this finding for yellows and reveal a similar situation to be true in non-agoutis. The pigment increase caused by the *dd*-for-*DD* substitution seems much more radical in a number of yellow genotypes than in sepias, but E. S. RUSSELL cautions that her data are not as accurate as could be desired. However, their general direction combined with our extract findings supports the view that the dopa reactions in *dd* types—originally thought to be an exception to the parallelism with yellow—merely correspond to a previously unknown increase in natural pigment. Since both eumelanin and phaeomelanin are probably affected, the dopa reaction here does not show a discriminatory parallelism with one or the other (certain detailed steps cannot be used in the analysis because of the inaccuracy of the natural pigment data in "dilutes"). The gene seems to have two other effects on the reaction (possibly correlated with the one just discussed): first, a considerable clumping of the dopa melanin produced (often so striking that the follicles seem to be bursting with solid masses of pigment); and second, an occasionally increased variability among the animal means within a genotype, and often of the various follicles of one animal (see table 2). *dd* could be postulated to have an effect on the distribution of the enzyme and also—possibly—on its active quantity.

The overall reduction of the dopa reaction by *pp* is found in 13 comparisons to be on the borderline of significance (P is very slightly above 0.01). In spite of the fact that, according to E. S. RUSSELL (1946), the pink-eye gene affects the pigmentation of *both* sepia and yellow hairs, the close correspondence of the dopa reaction with the latter type is here clear-cut. The degree of reduction of the dopa grades is very slight, paralleling the weak action of the gene on phaeomelanin and contrasting with its radical effect on eumelanin where all granule attributes are involved to a marked extent. One particular detail also supports the correspondence with yellow: in the dopa reaction, the greatest difference between *pp* and *PP* is found on the chinchilla background; this parallels our findings with extracts of yellow pigment as well as E. S. RUSSELL'S idea that the effect of *pp* on yellow occurs mainly at what she thinks may be a critical level at $A^{v}ac^{ch}c^{ch}pp$. Since $A^{v}ac^{e}c^{e}pp$ is pigment-free and albino series heterozygotes have not yet been studied by her, this idea of critical levels is not too firmly founded and one may instead postulate modifiers in the $c^{ch}c^{ch}pp$ stock. If such is the case, these modifiers must act equally on yellow pigment and the dopa reaction. However, in favor of the view that *pp* somewhat lowers the efficiency of the dopa reaction (and yellow?) in general and more so at critical levels are our results obtained with certain albino series compounds to be discussed when dominance relations in that series are considered.

The albino series was available in all its combinations on two backgrounds —*aa* and *aapp*—and almost complete on A^va, A^vapp, *aabb*, and A^vabb. All the other backgrounds (except *aadd* and A^vadd on which we had 3 combinations each) were represented only by *CC*. Wherever the series is complete or nearly so, its lower members are found to produce a gradual reduction in the reaction from the maximum in *CC* to nothing in c^ec^e. Considering at first only the homozygous albino series combinations, we can again point out the parallelism with yellow. Substituting $c^{ch}c^{ch}$ for *CC* causes a considerable drop in grade, comparable to the change by this substitution of yellow coats to cream, but different from the lack of effect of $c^{ch}c^{ch}$ on browns (*aabbc*$^{ch}c^{ch}$ is indistinguishable from *aabbCC*) and the weaker action on sepias (*aac*$^{ch}c^{ch}$, in terms of visual grades, only slightly lighter than *aaCC*). The particularly marked action of $c^{ch}c^{ch}$ on a pink-eye background has already been pointed out and discussed as being probably due to a critical level both in the dopa reaction and in yellow pigment. Extreme dilution, c^ec^e, in A^va animals produces white hairs. The dopa reaction threshold, however, seems to be slightly lower, since a faint reaction is obtained in *aac*$^ec^e$, $A^vac^ec^e$, *aabbc*$^ec^e$ and $A^vabbc^ec^e$. That these types are close to the threshold for yellow pigment, is shown by a few granules being occasionally found in them (see first column of table 1b). With c^ec^e on a *pp* background, not even the dopa melanin threshold is reached, in line with the general slight depression of efficiency postulated for the *p* gene. One rather striking point of yellow-parallelism comes out in connection with extreme dilute: *aapp* and *aac*$^ec^e$ are visually almost equal in intensity and have virtually the same total pigment volumes; yet, while the former gives a full dopa reaction, that of the latter is almost negligible, paralleling the conditions of natural pigment in A^vapp and $A^vac^ec^e$ respectively. All c^ec^e combinations are free of any kind of pigment and neither one of the two tested gave a dopa reaction.

A consideration of dominance relations (in dopa reaction as compared with natural pigment) within the albino series is made somewhat difficult by the fact that E. S. RUSSELL has not yet obtained measurements for albino series heterozygotes. We therefore have no basis for comparison except DUNN'S (1936) rough visual grades and our own, so far incomplete, extract data. It must also be remembered that no quantitative conclusions can be drawn as to dominance relations in the reaction, since our scale of grades is almost certainly not an arithmetic one as to actual quantities of dopa melanin formed. To consider first the dominance of *C*: this is generally assumed to be complete in all backgrounds and is found to be so with regard to the dopa reaction also, except probably with *pp*. If this incomplete dominance in the presence of *pp* is real, it is in accordance with the general depressing effect postulated for the pink-eye gene. Chinchilla is not dominant over extreme dilution and albinism, the two compounds being intermediate in their reactions to the respective homozygous types. In all combinations $c^{ch}c^e$ dopa grades are higher than $c^{ch}c^a$, paralleling the extract data for yellows and confirming again the view that there is some activity with c^e. Chinchilla combinations with brown bring out further evidence of the parallelism with yellow: as has already been mentioned, *aabbc*$^{ch}c^{ch}$ is indistinguishable from *aabbCC* by all criteria; in addi-

tion, DUNN (1936) finds *aabbc^{ch}c^{e}* as intense as these two types and we get the same impression from the gross appearance of the animal. The drop in dopa grade between *bbCC* and *bbc^{ch}c^{ch}* has already been pointed out; now an additional drop is found from *bbc^{ch}c^{ch}* to *bbc^{ch}c^{e}*. This is comparable to the difference in yellow pigment between the types apparent from our extract data as well as from the gross appearance of the heterozygote. *c^{e}c^{a}* was available only with *aa*, *A^{v}a* and *aapp*. In the first of these it appears that *c^{e}* is dominant in reaction. No conclusions can be drawn in the other two.

The last of the major color genes to be considered are *A^{v}* and *a*. In 14 possible comparisons we found no significant difference between the grades of

TABLE 5

Distribution and means of dopa reaction grades in W^{v} combinations.

	ww							*W^{v}w*							*W^{v}W^{v}*						
	0	1	2	3	4	5	MEAN	0	1	2	3	4	5	MEAN	0	1	2	3	4	5	MEAN
aapp	—	—	—	8	118	21	4.09	—	—	—	20	148	4	3.91	144	—	—	—	—	—	0.00
aabb	—	—	—	19	103	29	4.07	—	—	—	28	189	7	3.91	181	—	—	—	—	—	0.00
aabb	—	—	—	7	69	22	4.15	—	—	—	18	146	14	3.98	147	—	—	—	—	—	0.00
aa	48 bulbs (3–5); white spot 0.00																				
A^{v}a	—	—	—	1	143	5	4.03	—	—	—	8	94	3	3.95							

corresponding *A^{v}a* and *aa* genotypes. The reaction in sepias as well as yellows parallels the natural yellow series.

Of the four less well known series, only *W^{v}* combinations were graded by the usual method (table 5). Results for *ln* and *m* are stated qualitatively.

In table 5, each grade represents one animal, each line across the three main columns the segregants of one litter. No reaction occurs in *W^{v}W^{v}* or in the white spots of *W^{v}w* animals. The colored portions of *W^{v}w* combinations show a slightly lower reaction throughout than the corresponding *ww* types. A test of significance for the total of four comparisons gives P less than 0.01.

The dopa reaction in leadens produced solid black follicles, looking much the same as some *dd* types. Control bulbs showed the considerable degree of clumping expected from the similar condition previously reported for the hairs.

Misty hair bulbs on the other hand are quite normal in their granular appearance, with only very rare clumps. The dopa reaction also seemed full and normal with no undue clumping. Where misty was combined with *dd*, the "dilution" effect occurred without modification.

The white spot of one piebald animal was tested. It gave no reaction. Whether this was due to an absence of enzyme or melanoblasts cannot be determined by the present method.

CONCLUSIONS

Results of previous workers have been reviewed in some detail by W. L. RUSSELL (1939). We shall restrict ourselves to a comparison of our results with

those obtained in frozen sections and extracts by W. L. RUSSELL and GINSBURG respectively in the guinea pig.

Throughout the discussion of results we have stressed the fact that, although qualitatively the pigment produced resembles eumelanin, the quantitative effect of each gene substitution on the reaction in both yellows and non-agoutis closely parallels the effect on yellow pigment. Our results are, therefore, quite similar in principle to those obtained by W. L. RUSSELL (1939) and confirmed by GINSBURG (1944). The conclusion must be drawn that the dopa reaction measures some phase of the yellow producing system and that this system is present in sepias as well as in yellows.

The mouse is, in one or two respects, not such favorable genotypic material for this type of study as is the guinea pig. The albino series in the latter is more suitable for distinguishing between possible parallelism with the yellow and sepia systems since it has five alleles (as against four available ones in the mouse) affecting *ee* and *EE* backgrounds in a different order. In the mouse, the order in $A^{v}a$ and *aa* is the same and we must base our parallelism on the different magnitude of the steps. Another disadvantage of the mouse is the lack, so far, of a factor similar to *f* which reduces yellow pigment without by itself affecting sepia. Among the several practical advantages of the mouse must be mentioned the greater abundance and uniformity of follicles, making grading more reliable.

There are several direct points of comparison in the dopa reactions. As in the guinea pig, the white spots of *ss* animals fail to produce dopa melanin. Also as in the guinea pig, substitutions of *bb* for *BB* do not affect the reaction. A slightly different result was obtained for *pp*. In the guinea pig, this substitution was found not to cause a significant difference in dopa grades. In the mouse, pink-eye was the only one of the color genes, apart from the *C* series, which probably affected the reaction significantly. The effect, however, is slight and, as has already been discussed, it reconciles with yellow-parallelism.

There are other minor departures from the guinea pig results. In that animal, combinations with *ee* (yellow) gave slightly but significantly less reaction than the corresponding combinations with *E*-(sepia). This result was explained by WRIGHT (unpublished) and GINSBURG (1944) as possibly due to the fact that more of the yellow producing system is available for the dopa reaction in sepias where it is not actively engaged in producing yellow pigment than in *ee* types where it is. In the mouse, we found no significant difference between corresponding yellow ($A^{v}a$) and sepia (*aa*) genotypes. As already pointed out, yellow is dominant in the mouse and an allele of agouti; it is recessive and independent in the guinea pig. In this connection we should consider the suggestion of BAKER and ANDREWS (1944) and of NICKERSON (1946) that yellow "melanin" is an oxidation product of natural black melanin and of dopa melanin. Such a relationship should produce two results as far as the physiological genetic work is concerned: (1) since presumably more oxidizing enzyme is present in yellow genotypes, the dopa reaction in those types should be higher; actually, it is lower in the guinea pig and equal in the mouse. (2) The dopa reaction in genotypes showing much oxidase activity (that is, giving a grade approaching 4 or

higher) should produce a redder type of melanin than the reaction in weak types; actually, the colors appear alike under the microscope. In other words, levels of oxidative activity seem to be expressed in quantity rather than quality of pigment. If black-yellow differentiation were merely a matter of one quantitative step, it might also be questioned why different gene substitutions (for example *pp*) act so differently in the two systems. The conclusions of BAKER and ANDREWS and of NICKERSON are based on the slopes of light absorption curves of the various melanins; in the case of such colloidal solutions, these cannot provide more than an approximate criterion.

There is one further minor difference between the dopa reaction results of the mouse and the guinea pig. In the former there is evidence for a slightly higher threshold for yellow pigment production than for the formation of dopa melanin: we find a weak reaction in three $A^{v}a$ whites as well as their corresponding *aa* types. In the guinea pig, this situation does not seem to exist: W. L. RUSSELL (1939) found no dopa melanin produced in any of the *ee* white types and the weak reaction in two of the corresponding *EE*'s might have been expected from the slightly increased reaction in sepia types in general. Whether, and if so how, this difference between the species is correlated with the fact that in one (the guinea pig) the reaction is significantly lower in yellows while in the other the reaction is the same in the two backgrounds cannot be stated without more information. (One might postulate, for example, that in the guinea pig the yellow producing enzyme is more easily tied up than in the mouse.)

The rather consistent increase in dopa melanin production by Maltese dilution as well as the effects of the miscellaneous coat color genes W^{v}, *ln* and *m* have no homologues in the guinea pig.

The paramount fact emerging from the study of both animals is the parallelism of the production of dopa melanin with that of pigment in the phaeomelanic types—a relationship which holds down to rather fine details when the frozen section method is employed. The fact that the parallelism occurs in *aa* as well as $A^{v}a$ types makes two alternative interpretations possible with regard to the nature of sepia-yellow differentiation:

(1) It could be postulated that sepia-yellow differentiating genes act to produce different substrates and that the enzyme system (controlled by other genes) which acts on these substrates is alike in sepias and yellows and produces different results due only to the difference in the raw material. The possible nonspecificity of the enzyme has been discussed in the introduction to this paper. NICKERSON (1946) points out that the pigments produced are known to vary according to the substrate utilized. If there be a common precursor of the eu- and phaeomelanic substrates, it can be postulated: (a) that the sepia-yellow differentiating genes change it into two different substances in the two types, or (b) that it is changed only in one type and utilized unchanged in the other. Dopa is either the yellow substrate or a substance quite similar to it. (The fact that dopa melanin itself appears sepia rather than yellow may be due to a variety of physical or chemical reasons.) The yellow gene may thus have a positive action in changing a common precursor to dopa or a dopa-like

substance; or dopa (or a dopa-like substance) may be a common precursor transformed to another immediate substrate in sepia types. There is no clue as to whether to look for a simpler or more complicated substance as being the immediate sepia substrate, since the action of the yellow gene (in the former case) or of the sepia gene (in the latter) may be either breakdown or elaboration. The discovery of another chromogen whose effect paralleled sepia both in yellow and sepia genotypes would prove the postulate that the sepia-yellow difference was one of substrates rather than enzyme systems, and the relation between this chromogen (presumably akin to the sepia substrate) and dopa would provide a chemical clue as to the action of sepia-yellow differentiating genes.

(2) It is possible that the black-yellow differentiating genes produce some condition, such as pH, redox potential, etc., which acts as a trigger mechanism calling one of two possible co-existing modes of gene-enzyme action into play on a single substrate, perhaps dopa or a substance similar to it. The conditions of the dopa reaction must then approximate those produced by the yellow gene. This scheme is not quite as simple as the first and requires more than single gene effects.

The scheme presented by WRIGHT (1942) is based primarily on the postulated order and interaction of gene effects rather than on hypotheses as to the specific biochemical action of each gene. Both of the above interpretations are reconcilable with the essential parts of WRIGHT's scheme in this respect. Their primary emphasis lies, however, on possible biochemical mechanisms of one specific underlying action—black-yellow differentiation. It will be easier to speculate on the specific actions of other genes once this fundamental differentiation into pigments is understood (RUSSELL *et al.* 1948).

SUMMARY

1. Frozen sections of mouse skins of 73 genotypes were incubated in buffered dopa solutions according to the technique of W. L. RUSSELL (1939) which produces uniform results within each genotype.

2. The results are in principle similar to those found in the guinea pig. Qualitatively, the pigment produced resembles eumelanin, but the quantitative effect of each gene substitution on the reaction in both yellows and non-agoutis parallels the effect on yellow pigment:

a. *bb* does not affect the reaction.

b. *pp* lowers the reaction slightly but probably significantly. That there is a similar slight reducing effect of the gene on yellow pigment is shown by E. S. RUSSELL's recent data (1946).

c. The lower combinations of the *C* series produce reductions, matching their effect on yellows more closely than on non-agoutis.

If the dopa reaction indicates the presence of an enzyme system, it must be concluded that substitutions at the *C* and possibly *P* locus act on natural yellow pigment through this enzyme system.

3. *dd* causes a rather consistent, though not significant, increase of the reaction. Recent measurements of natural pigment reveal an increase by *d* of the

total pigment volume in yellows and probably also sepias. The dopa reaction here thus does not show a discriminatory parallelism with one or the other type on the basis of present evidence.

The *d* gene which produces clumping of natural pigment also causes the dopa melanin to be formed in clumps. It may, therefore, be postulated to affect the intracellular distribution of the enzyme system in addition to any effect it may have on the system's active quantity.

4. There is no significant difference in the reactions of $A^{v}a$ and corresponding *aa* types—a slight departure from the guinea pig results.

5. No dopa reaction occurs in the white spot of an *ss* animal.

6. $W^{v}w$ dilutes the reaction slightly, but significantly. $W^{v}W^{v}$ prevents it completely, as does the white spot of a $W^{v}w$ animal.

7. The effects of treating sections from misty and leaden animals by our method were observed qualitatively. The former gene is without effect on the reaction; the latter causes clumping of natural pigment as well as of dopa melanin, similar to *dd*.

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LITERATURE CITED

ADAMS, M. H., and J. M. NELSON, 1938 On the nature of the enzyme tyrosinase. J. Amer. Chem. Soc. **60**: 2474–2478.

BAKER, M. R., and A. C. ANDREWS, 1944 The melanins. I. Studies of the hair pigments of the guinea pig. Genetics **29**: 104–112.

BLOCH, B., 1927 Das Pigment. Handbuch der Haut- und Geschlechtskrankheiten I(1): 434–541.

BRAUCH, L. R., and W. L. RUSSELL, 1946a A study of the physiological genetics of coat color in the mouse by means of the dopa reaction. (Abstract.) Genetics **31**: 212.

1946b Colorimetric measurement of the effects of the major coat color genes in the mouse on the quantity of yellow pigment in extracts. (Abstract.) Genetics **31**: 212.

DANNEEL, R., 1936 Die Färbung unserer Kaninchenrassen und ihre histogenetischen Grundlagen, Z.I.A.V. **71**: 231–264.

DANNEEL, R., and K. SCHAUMANN, 1938 Zur Physiologie der Kälteschwärzung beim Russenkaninchen. III. Die von dem Erbfactor a_n gesteuerte Fermentbildung in der Unterkühlungsphase. Biol. Zbl. **58**: 242–260.

DUNN, L. C., 1936 Studies of multiple allelomorphic series in the house mouse. I. Description of agouti and albino series of allelomorphs. J. Genet. **33**: 443–453.

1934 Analysis of a case of mosaicism in the house mouse. J. Genet. **29**: 317–326.

DUNN, L. C., and W. EINSELE, 1938 Studies of multiple allelomorphic series in the house mouse. IV. Quantitative comparisons of melanins from members of the albino series. J. Genet. **36**: 145–152.

DURHAM, F. M., 1904 On the presence of tyrosinases in the skins of some pigmented vertebrates. Proc. Roy. Soc. **74**: 310–313.

DU SHANE, G. P., 1935 An experimental study of the origin of pigment cells in Amphibia. J. Exp. Zool. **72**: 1–32.

EASTLICK, H. L., 1939 The point of origin of the melanophores in chick embryos as shown by limb bud transplants. J. Exp. Zool. **82**: 131–150.

EINSELE, W., 1937 Studies of multiple allelomorphic series in the house mouse. II. Methods for the quantitative estimation of melanin. J. Genet. **34**: 1–18.

FIGGE, F. H. J., 1940 Pigment metabolism studies. The regulation of tyrosinase melanin formation by oxidation-reduction systems. J. Cell. Comp. Physiol. **15**: 233–247.

GINSBURG, B., 1944 The effects of the major genes controlling coat color in the guinea pig on the dopa oxidase activity of skin extracts. Genetics **29**: 176–198.

GRÜNEBERG, H., 1943 The Genetics of the Mouse, pp. 22–49. Cambridge: At the University Press.

HEIDENTHAL, G., 1940 A colorimetric study of genic effect on guinea-pig coat color. Genetics **25**: 197–214.

HOGEBOOM, G. H., and M. H. ADAMS, 1942 Mammalian tyrosinase and dopa oxidase. J. Biol. Chem. **145**: 273–279.

KEILIN, D., and T. MANN, 1938 Polyphenol oxidase: purification, nature, and properties. Proc. Roy. Soc. (B) **125**: 187–203.

KRÖNING, F., 1930 Die Dopareaktion bei verschiedenen Farbenrassen des Meerschweinchens und des Kaninchens. Arch. EntwMech. **121**: 470–484.

KUBOWITZ, F., 1938 Re-synthese der Phenoloxydase aus Protein und Kupfer. Biochem. Z. **296**: 443.

LITTLE, C. C., 1913 Experimental studies of the inheritance of color in mice. Carnegie Inst. Wash. Publ. **179**: 11–102.

NICKERSON, M., 1946 Relation between black and red melanin pigments in feathers. Physiol. Zoöl. **19**: 66–77.

ONSLOW, H., 1915 A contribution to our knowledge of the chemistry of coat-colour in animals and of dominant and recessive whiteness. Proc. Roy. Soc. (B) **89**: 36–58.

PARKINSON, G. G., and J. M. NELSON, 1940 On the nature of the enzyme tyrosinase. J. Amer. Chem. Soc. **62**: 1693–1697.

PUGH, C. E. M., 1933 Tyrosinase from the skin of certain black rabbits. Bio-Chem. J. **27**: 475–479.

RAPER, H. S., 1928 The aerobic oxidases. Physiol. Rev. **8**: 245–282.

1932 Tyrosinase. Ergebn. Enzymforsch. **1**: 270–279.

RAWLES, M. E., 1940 The development of melanophores from embryonic mouse tissues grown in the coelom of chick embryos. Proc. Nat. Acad. Sci. **26**: 673–680.

1947 Origin of pigment cells from the neural crest in the mouse embryo. Physiol. Zöol. **20**: 248–266.

REED, S. C., 1938a Determination of hair pigments. II. Transplantation results in mice, rats and guinea pigs. J. Exp. Zool. **79**: 331–336.

1938b Determination of hair pigments. III. Proof that expression of the black-and-tan gene is dependent upon tissue organization. J. Exp. Zool. **79**: 337–346.

ROTHMAN, S., 1942a In vitro studies on pigmentation. I. The oxidation of tyrosin by ultraviolet irradiation. J. Invest. Dermatol. **5**: 61–67.

1942b In vitro studies on pigmentation. II. Influence of ascorbic acid on oxidation of tyrosin by ultraviolet irradiation. J. Invest. Dermatol. **5**: 67–75.

RUSSELL, E. S., 1939 A quantitative study of genic effects on guinea-pig coat colors. Genetics **24**: 332–355.

1946 A quantitative histological study of the pigment found in the coat-color mutants of the house mouse. I. Variable attributes of the pigment granules. Genetics **31**: 327–346.

1946a A quantitative study of genic effects on coat color in the house mouse. Genetics **31**: 227.

1948 A quantitative histological study of the pigment found in the coat-color mutants of the house mouse. II. Estimates of the total volume of pigment. Genetics **33**: 228–236.

RUSSELL, W. L., 1939 Investigation of the physiological genetics of hair and skin color in the guinea pig by means of the dopa reaction. Genetics **24**: 645–667.

RUSSELL, W. L., E. S. RUSSELL, and LIANE R. BRAUCH. 1948 Problems in the biochemistry and physiological genetics of pigmentation in mammals. Spec. Publ. N. Y. Acad. Sci. **4**: 447–453.

SCHILLING, L., 1939 Untersuchung zur Variabilität der Meerschweinchen haare und ihrer Pigmente. Z.I.A.V. **76**: 533–569.

SCHULTZ, W., 1925 Verhalten der einzelnen Färbungsgene zur Dopareaktion bei Kaninchenrassen. Arch. EntwMech. **105**: 677–710.

SPIEGEL-ADOLF, M., 1937 Studies on melanins. I. Photosynthetic melanins. Biochem. J. **31**: 1303–1310.

WERNEKE, F., 1916 Die Pigmentierung der Farbenrassen von *Mus musculus* und ihre Beziehung zur Vererbung. Arch. EntwMech. **42**: 72–106.

WILLIER, B. H., 1941 An analysis of feather color pattern produced by grafting melanophores during embryonic development. Amer. Nat. **75**: 136–146.

WOOLLEY, G. W., 1941 "Misty" a new coat color dilution in the mouse, *Mus musculus*. Amer. Nat. **75**: 507–508.

WRIGHT, S., 1927 The effects in combination of the major color-factors of the guinea pig. Genetics **12**: 530–569.

1941 A quantitative study of the interactions of the major colour factors of the guinea pig. Proc. Seventh Int. Genetical Cong., pp. 319–329.

1942 The physiological genetics of coat color of the guinea pig. Biol. Symp. **6**: 337–355.

WRIGHT, S., and O. N. EATON, 1926 Mutational mosaic coat patterns of the guinea pig. Genetics **11**: 333–351.

THE FREQUENCIES OF TWINS, RELATIVE TO AGE OF MOTHERS, IN AMERICAN POPULATIONS

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THE frequency of twin births varies in different populations. Furthermore, it is known for several European groups, for the white population of Australia and for Japanese that a relation exists between the age of the mother and the percentage of twin births. An analysis of vital statistics data by various authors has led to the conclusion that dizygotic and monozygotic twins behave differently in this respect (see DAHLBERG 1926; KOMAI and FUKUOKA 1936). In the white populations analyzed the frequency of dizygotic twins rises steadily with increasing age of the mother until a maximum is reached in the 35–40 year age group. The maximum frequency was found to be about three times that of the initial frequency. After the maximum the curve declines again. For Japanese, the data are relatively limited but they indicate a similar trend as in whites, except for the maximum occurring slightly earlier, at age 34. In contrast to the distinct correlation between age of mother and frequency of dizygotic twins there is little variation between different age groups of mothers in frequency of monozygotic twins. In various populations there appears a slight increase with rising age of mothers but the maximum increase, as seen in data fom Australia, is less than 30 percent over the initial frequency.

No extensive analyses of the vital statistics of plural births had been available for the United States until the recent publications by STRANDSKOV and his collaborators (1945–1946). These studies report on the gross plural birth frequencies in the total, the "white" and the "colored" U. S. populations, on the sex ratios among them, and, in the last cited report, on the frequencies of monozygotic and dizygotic twin births.

Data on the relation between age of mothers and twin births in the United States have been published by the U. S. BUREAU OF THE CENSUS. They are not suitable for a detailed treatment because the figures are neither broken down for different racial groups nor do they give the sex of twins born. The former information is important since it is known that differences exist in total frequencies of twin births for "white" and "colored" U. S. populations, the latter information is needed for an estimate of the partition of gross twin frequencies into dizygotic and monozygotic ones. The BUREAU OF THE CENSUS is in possession of the required raw data, and DR. HALBERT L. DUNN, Chief of the NATIONAL OFFICE OF VITAL STATISTICS, has kindly made available to us a table giving the number of cases of twins by age of mother, sex, and race in the United States for the year 1938. The listing is according to five year age groups

of mothers, and includes only those twin births in which at least one twin was born alive. Racially the entries are subdivided into "whites," "negroes" and "other races." The term "white" includes any members of the Caucasoid group. The term "negro" refers to African negroes regardless of their admixture of Caucasoid or Mongoloid ancestry. Whites and negroes alone will be considered (table 1) since the total number of twin births among "other races" is too small,

TABLE 1

*Number of all * confinements and of twin confinements, with at least one live born, by age of mother, sex, and race. United States 1938. Compiled by Federal Security Agency, U. S. Public Health Service, National Office of Vital Statistics.*

	TOTAL TWIN CONFINEMENTS	AGE OF MOTHERS (in years)									
		10–14	15–19	20–24	25–29	30–34	35–39	40–44	45–49	50–54	NOT STATED
White											
Total Confinements			229,007	628,347	547,077	338,583	176,391	56,953			
♂♂ Both Living	6,840	2	452	1,762	1,900	1,539	916	238	24	1	6
1 Living	570		51	137	146	120	92	24			
♂♀ Both Living	6,781	1	264	1,419	2,025	1,695	1,095	263	9		10
1 Living, ♂	191		16	33	52	47	28	15			
1 Living, ♀	266		11	44	68	68	60	13			
♀♀ Both Living	6,726		490	1,757	1,921	1,426	854	255	16		7
1 Living	435		40	122	105	83	62	21	1		1
Total White	21,809	3	1,324	5,274	6,217	4,978	3,107	831	50	1	24
Negro											
Total Confinements			65,747	81,963	54,255	31,510	21,424	6,022			
♂♂ Both Living	1,026	3	131	261	261	167	156	38	6	1	2
1 Living	148		24	32	37	30	19	6			
♂♀ Both Living	1,162		113	285	308	241	181	29	5		
1 Living, ♂	72		7	16	14	23	12				
1 Living, ♀	93		12	31	21	14	14	1			
♀♀ Both Living	1,049		138	284	243	193	154	33	2	1	1
1 Living	129		17	35	36	21	17	3			
Total Negro	3,679	3	442	944	920	689	553	110	13	2	3

* For a qualification see text.

namely 156, to justify an analysis. For the same reason we have also omitted a discussion of all data relating to mothers less than 15 years and 45 or more years old.

In order to estimate the number of dizygotic and monozygotic among the total number of twin births, WEINBERG's differential method (1902) has been applied. It is based on the fact that all unlike sexed twins are undoubtedly dizygotic, and on the expectation that the number of like sexed dizygotic twins should bear a simple relation to that of the unlike sexed ones. Given a sex ratio of females to males in the proportion of p:q, dizygotic twins should be distributed as follows: ♀♀ : ♀♂ : ♂♂ = p^2 : 2pq : q^2. In a population in which

$p=q=\frac{1}{2}$ this distribution takes the form $\frac{1}{4}:\frac{1}{2}:\frac{1}{4}$, that is, the total number of dizygotic twins is twice that of the observed number of unlike sexed twins. The number of monozygotic births is obtained by subtracting that of the dizygotics from the total. In many populations the sex ratio deviates from equality. It is therefore more correct to calculate the number of dizygotic twins by means of the actual sex ratio p:q instead of the ideal 1:1 ratio. This is done by equating the number of different sexed twins to 2pq of the total of dizygotics. A still more accurate approach might make use not of the sex ratio of the population as a whole but only of that of the twin population itself. At least two different methods suggest themselves. One may either use the over-all sex ratio of the twin population, or, in an investigation in which the twin populations are sub-

TABLE 2

Sex ratios among twin confinements with at least one living, by age of mothers.

	AGE OF MOTHERS					
	15–19	20–24	25–29	30–34	35–39	40–44
White	0.48980	0.50190	0.50161	0.51507	0.51481	0.49158
	0.51020	0.49810	0.49839	0.48493	0.48520	0.50842
Negro	0.50000	0.48623	0.51033	0.48766	0.50362	0.53636
	0.50000	0.51377	0.48967	0.51234	0.49638	0.46364

Sex ratio of all twin confinements with at least 1 living	*white*	.50399	.49601
	negro	.49946	.50054
Sex ratio of whole live birth population	*white*	.51367	.48633
	negro	.50552	.49448

divided into age groups, one may use, for each group the sex ratio of its specific twin population. This latter procedure is perhaps the most appropriate one but it is subject to a greater statistical error of the various sex ratios employed on account of the smaller size of the twin groups as compared with the twin population as a whole.

STRANDSKOV and EDELEN (1946) have shown that for U. S. twin populations the estimates for dizygotic and monozygotic twin births yield very similar figures whether the sex ratio of the whole live birth population or that of the twins only is used. We have treated the data in two different ways. For one set of our calculations we have employed equally for all groups of twins of a given racial population the sex ratio of its whole live birth population, that is, the ratio based on the largest total of events. In another set, we have employed for the treatment of each age-group of twins its own specific sex ratio. The various sex ratios, as derived from the data of table 1, are given in table 2.

In the census reports on VITAL STATISTICS OF THE UNITED STATES the word "birth" in singular is used in two different meanings. In some cases it refers to the event which brings forth an individual child, in others it applies to the

product of labor regardless of the number of children born. Thus, "total live births" comprise all individual live children born regardless of whether in single or plural births, while "number of twin births" states the totality of labors which resulted in two children with one or both born alive. If one is concerned with the relative frequency of twin births, the question is obviously that of the fraction of twin confinements among all confinements. In order to find this fraction the number of all confinements must be known. As pointed out, this number is not identical with that of the live births as given by the census data.

TABLE 3

Estimated percentages of monozygotic and dizygotic twin confinements with at least one living, among all† confinements with at least one living, for white and negro, according to age of mothers.

TYPE OF TWIN CONFINEMENTS	ESTIMATE BASED ON SEX RATIOS OF	AGE OF MOTHERS					
		15–19	20–24	25–29	30–34	35–39	40–44
Whites							
dizygotic	total white population	0.254	0.476	0.784	1.070	1.342	1.029
	age class of twins	0.254	0.476	0.785	1.070	1.342	1.030
monozygotic	total white population	0.324	0.363	0.352	0.401	0.419	0.430
	age class of twins	0.324	0.363	0.352	0.400	0.419	0.429
Negroes							
dizygotic	total negro population	0.402	0.811	1.265	1.766	1.933	1.002
	age class of twins	0.402	0.810	1.265	1.765	1.933	0.996
monozygotic	total negro population	0.271	0.341	0.431	0.428	0.649	0.825
	age class of twins	0.271	0.342	0.431	0.422	0.649	0.830

† For a qualification see text.

It may be derived from this number by subtracting from it the number of twin confinements which resulted in two live children. It would be desirable to make further adjustments by taking account of triplet and quadruplet confinements. This is not possible since the available data do not give information on the distribution over the various age groups, of higher plural confinements. They could not be sorted out of the "total live births" where they are included, and appear in our compilations as if they consisted of as many separate confinements as live individuals were born. The resulting error, an overestimate of total confinements, is small since there were only 232 triplet confinements among whites, resulting in 597 live born children, and 48 triplet confinements among "all other races" (presumably mostly negroes) resulting in 127 live born children. Only two quadruplet confinements occurred among whites, with a total of four children surviving, and none among negroes. In summary, the term "frequency of twin confinements among all confinements" as used in this report refers to the number of twin confinements resulting in at least one live child divided by the total number of confinements giving birth to at least one

live child. It may be added that earlier authors have not always made clear the definitions on which their treatment of twin data was based.

The calculated frequencies of dizygotic and monozygotic confinements as percentages of all live birth confinements are listed in table 3, and graphically

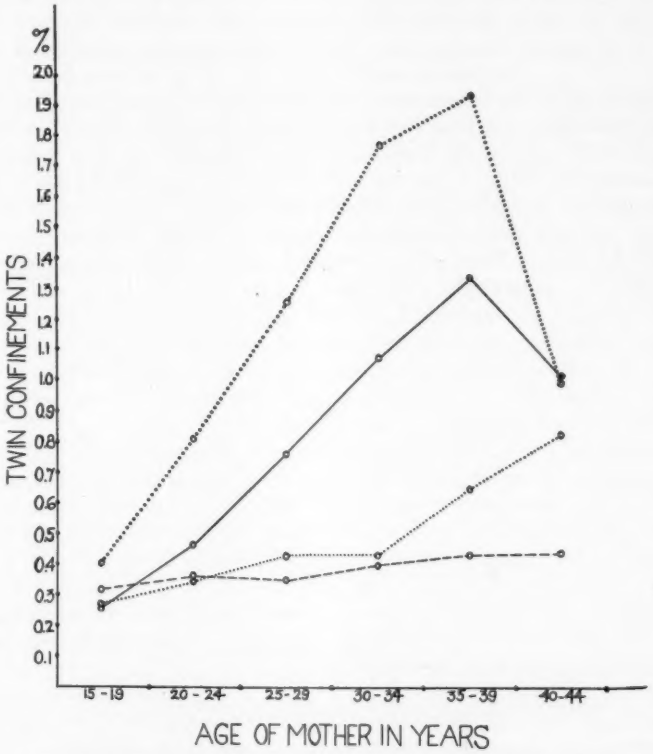

FIGURE 1. Frequency of twin confinements in relation to age of mothers.
.... dizygotic, negroes
—— dizygotic, whites
···· monozygotic, negroes
---- monozygotic, whites

represented in figure 1. It is seen from the table that the two sets of estimates, based on the two different types of sex ratios employed, are nearly identical. They may therefore be considered together.

The dizygotic confinements for whites show the familiar rise in frequency from the youngest age class of mother until the 35–39 year class and the drop subsequently to this age. Thus, the whites of the United States follow the same trend as the white populations of Europe and Australia as well as the less

accurately known trend of the Japanese. The dizygotic twin births for negroes also increase in frequency from the class of youngest mothers to that of group 35–39 years, and drop sharply in the following age class. Negroes have a higher total frequency of dizygotic twin confinements than whites. The age-frequency curve shows that the initial twin frequency for negroes lies higher than that for whites, and that this higher level is maintained until both polygons have reached their maximum. The rate of increase in dizygotic twin frequency with age of mothers in general is higher for negroes than for whites so that the absolute differences in frequencies increase with each age class up to 30–34 years. After the peak has been reached, the values for the two racial groups become nearly identical, the decline in frequency being greater for negroes than for whites. A test was made of the significance of the racial difference in decline, the null hypothesis being that $q_{yw}/q_{ow} = q_{yn}/q_{on}$ where the q's are the proportions of confinements leading to unlike sexed twins among younger whites (35–39 years), older whites (40–44 years), and younger and older negroes, respectively. Chi square[1] is 2.80 with one degree of freedom, corresponding to a P value just below 0.10. It is, therefore, not unlikely that the greater decline for negroes in proportion of dizygotic twin confinements between these two age groups is a reflection of a real difference between negroes and whites and not due to random sampling variations. However additional data are needed to establish, or rule out, this point.

The data for frequencies of monozygotic twin confinements for both whites and negroes with one exception each show a steady rise from the youngest to the oldest age class of mothers within the range investigated. The exceptional drop in each racial series, from 0.363 to 0.352 among whites and from 0.431 to 0.422 among negroes is not significant statistically. A rank correlation coefficient after KENDALL (1938) between frequency of monozygotic twin confinements and maternal age yields a value for each of the two racial populations of 0.867 which is statistically significant, the standard error being 0.3549 and the probability level 0.02.

The rank correlation coefficient is rather an indication of the existence of a correlation between the order of the variable twin confinements and the objective order of the age sequence than a full evaluation of the *amount* of variation within the order of variables. It is obvious from an inspection of the polygons for monozygotic twin confinements that the actual significance of the increase in frequency of confinements with age of mothers is much greater among the

[1] The following approximate expression for chi square was obtained by MISS ELIZABETH L. SCOTT of the Statistical Laboratory, UNIVERSITY OF CALIFORNIA, using a method of NEYMAN (1940):

$$\chi^2 = \frac{(q_{yn}q_{ow} - q_{yw}q_{on})^2}{\frac{q_{on}^2}{m_{yw}} + \frac{q_{yw}^2}{m_{on}} + \frac{q_{ow}^2}{m_{yn}} + \frac{q_{yn}^2}{m_{ow}}}$$

where

$$m_{ij} = \frac{N_{ij}}{q_{ij}(1 - q_{ij})},$$

N_{ij} being the total number of confinements in a given age and racial class.

negro population than among the whites. For the latter the maximum rise in frequency is from 0.324 to 0.430 that is only 33 percent of the initial value, for the former it is from 0.271 to 0.830, or 210 percent. As a further test of the reality of rise in monozygotic twin confinements with age of mother, the frequencies of these confinements among mothers of age 15–29 and 30–44 were compared with each other (table 4). For both whites and negroes the probabilities are highly in favor of a significant difference between the two age groups.

TABLE 4

Number of estimated monozygotic twin confinements with at least one live born and of total confinements.

AGE OF MOTHERS	WHITES		NEGROES	
	MONOZYGOTIC TWIN CONFINEMENTS	TOTAL CONFINEMENTS	MONOZYGOTIC TWIN CONFINEMENTS	TOTAL CONFINEMENTS
15–29	4,947	1,404,431	692	201,965
30–44	2,339	571,927	322	58,956
Difference of ratios		0.000567		0.002035
Standard error of diff.		0.0001837		0.0006674
Ratio		3.09		3.05
P		0.002		0.002

It should be noted that the sampling errors of all our estimates of numbers of monozygotic twins are compounded of errors in the number of like sexed twins as well as in the number of unlike sexed twins. Assuming a sex ratio of 1:1 (it having been shown that small variations in sex ratio have a negligible effect on the estimated monozygotic twin numbers), the computed number of monozygotic confinements is simply the difference between the number of like sexed and the number of unlike sexed twin confinements. In comparing two groups, then, the null hypothesis is that $(p-q)-(p'-q')=0$, where p and q are the proportions of like and unlike sexed twin confinements in one group and p' and q' are the same proportions for the other group. Our estimate of this difference is:

$$\frac{m_1 - m_2}{M} - \frac{m_1' - m_2'}{M'}$$

where m_1 and m_2 are the actual numbers of like and unlike sexed twin confinements and M is the total number of confinements. We owe the following expression for an unbiased estimate of the sampling variance of the difference to PROF. ERICH LEHMANN of the Statistical Laboratory of the UNIVERSITY OF CALIFORNIA:

$$\tilde{V} = \frac{m_1 + m_2}{M^2} + \frac{m_1' + m_2'}{(M')^2} - \frac{(m_1 + m_1' - m_2 - m_2')^2 - (m_1 + m_1' + m_2 + m_2')}{(M)(M')(M + M' - 1)}.$$

With our data the last term of this expression is negligible.

The slight rise in frequency of monozygotic confinements among American whites has been met before, though less regularly than in our material, in data on other white populations. The striking rise among negroes, however, is a phenomenon unsuspected heretofore. Since the belief has often been expressed

that the frequency of monozygotic twinning is widely independent of racial constitutions, it may be justified to compare the two sets of data on whites and negroes point by point for each age group (table 5). Such a comparison shows that there is a rather high probability for the differences between the frequencies in the 35–39, and 40–44 year groups being significant. No significance can be attached to the differences between the younger age groups. That there is an overall difference between the trend of frequencies of monozygotic twin confinements in whites and negroes is suggested by a consideration of the first line of table 5. It is seen that the amounts of the differences between monozygotic twin confinements of the two racial groups follow a decreasing natural order, from +0.00053 for the youngest mothers to −0.00395 for the oldest, with only one exception in the 30–34 year group. The coefficient of rank correlation between the objective order of increasing age of mother and the order

TABLE 5

The probabilities that the differences between frequencies of monozygotic twin confinements with at least one living, for white and negroes, are due to random sampling only.

	AGE OF MOTHERS					
	15–19	20–24	25–29	30–34	35–39	40–44
Diff. of frequencies	+0.00053	+0.00022	−0.00079	−0.00027	−0.00230	−0.000395
Standard error of diff.	0.000357	0.000392	0.000577	0.000859	0.001142	0.001814
Ratio	1.48	0.56	1.37	0.31	2.01	2.18
P	0.14	0.58	0.17	0.76	0.04	0.03

of the differences between whites and negroes is 0.867, with the standard error being 0.3549 and the probability level 0.02. It is, therefore, reasonable to conclude that the observed relationship is not due to random errors of any sort.

The causes for the rise in frequencies of monozygotic twin confinements with age of mothers may be sought for either in circumstances favoring the occurrence of twinning in developing eggs, or in conditions permitting a higher number of monozygotic twin pregnancies to be brought to term, or in a combination of both of these factors. That a higher survival rate may play some role has been suggested by DAHLBERG (1926) who analyzed French data on frequency of stillbirths of twins according to age of mothers. He found that the percentages, in relation to all stillbirths, of monozygotic twin births with one or both twins stillborn show a sharp decrease with rising age of the mother. On the basis of this finding he pointed out that an inclusion of the monozygotic twins with one or two stillborn would partly counterbalance the slight increase with age of mother seen in twins with both liveborn. DAHLBERG concluded (p. 47): "Assuming that the stillborn were a far larger group than here found, the result of an inclusion of this finally might be to defeat the increase with age of the monozygotic in the total material . . . ."

Our own data are not strictly comparable to those of DAHLBERG's. We possess no figures on the distribution, according to age of mother, of twin confine-

ments with both twins stillborn. Such statistics are available for twin confinements with *one* stillborn. They may be compared with similar data which can be extracted from DAHLBERG's table 6. For the French population treated in this table it can be shown that no clear trend with age of mother exists regarding the frequencies of monozygotic twin confinements with one stillborn. If the

TABLE 6

Frequencies of twin confinements with one stillborn.

	AGE OF MOTHERS	
	15–29	30–44
France (1907–1910)*		
Total stillbirths	76,729	60,572
Monozygotic twin confinements with 1 stillborn	723	573
Monozygotic twin confinements with 1 stillborn in % of all stillbirths	0.94	0.95
United States (1938)‡†		
Whites		
Total stillbirths	32,895	11,628
Monozygotic twin confinements with 1 stillborn	500	312
Monozygotic twin confinements with 1 stillborn in % of all stillbirths	1.52	2.68
Negroes		
Total stillbirths	20,161	4,465
Monozygotic twin confinements with 1 stillborn	144	61
Monozygotic twin confinements in % of all stillbirths	0.71	1.37

* Calculated from DAHLBERG (1926).

‡ Excl. Massachusetts and New Hampshire.

† A 1:1 sex ratio has been assumed for purposes of calculating the frequency of monozygotic twins.

material is grouped into two age groups only, namely 15–29 and 30–44 years, the frequencies of monozygotic confinements with one stillborn become nearly exactly alike, being 0.94 and 0.95 percent respectively (table 6). In contrast to this the American populations, both whites and negroes, show a decided increase of twin confinements with one stillborn with rising age of mothers. For whites the increase is from 1.52 to 2.68 percent, for negroes from 0.71 to 1.37 percent. In the French population the relative stability in frequency of twin confinements with one stillborn is accompanied by a decrease with age of mothers in frequency of confinements with both stillborn. As stated before, for the American populations nothing is known about the latter group relative to the age of mothers. Only if the frequency of twin pairs with both stillborn decreased, and to such an extent as to outweigh the striking increase of twin

confinements with one stillborn, would the rising curve for the frequency of twin confinements with at least one liveborn become depressed and perhaps made horizontal. This, to us, seems improbable. We are, therefore, inclined to regard the rise in frequency of monozygotic twin confinements, demonstrated in our data both of whites and negroes as the expression of a variable in the maternal organism responsible for favoring the occurrence of twinning in developing eggs. New data, on twin abortions and stillbirths, are needed to test this conclusion.

SUMMARY

Data were analyzed regarding the white and negro births for the year 1938 in the United States of America:

1. For both whites and negroes the frequency of dizygotic twin confinements with at least one liveborn relative to the total number of confinements varies with the age of mothers. There occurs a steady increase until the 35–40 year class, and a sharp decrease in the 40–44 year class.

2. For both whites and negroes the frequencies of monozygotic twin confinements show a trend toward increase with age of mothers over the whole range of years. This trend is slight for whites, and striking for negroes. It is statistically significant for both populations.

3. A significant difference seems to exist between whites and negroes for the trend of increase with age of mothers of monozygotic twin confinements.

4. It is suggested that the increasing frequency of monozygotic twin confinements with age of mother is a reflection of factors favoring the occurrence of twinning in developing eggs.

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LITERATURE CITED

Dahlberg, G., 1926 Twin births and twins from a hereditary point of view. 296 pp. Stockholm.

Dunn, H. L. (Edit.), 1940 Vital Statistics of the United States. Part I. 583 pp. Washington.

Kendall, M. G., 1938 A new measure of rank correlation. Biometrica **30**: 81–93.

Komai, T., and Fukuoka, G., 1936 Frequency of multiple births among the Japanese and related peoples. Am. J. Phys. Anthrop. **21**: 433–447.

Neyman, J., 1940 Conception of equivalents in the limit of tests and its application to certain λ- and χ^2-tests. Ann. Math. Statist. **11**: 477–478.

Strandskov, H. H., 1945 Plural birth frequencies in the total, the "white" and the "colored" U. S. populations. Am. J. Phys. Anthrop. N.S. **3**: 49–55.

Strandskov, H. H., and Edelen, E. W., 1946 Monozygotic and dizygotic twin birth frequencies in the "white" and the "colored" U. S. populations. Genetics **31**: 438–446.

Strandskov, H. H., and Siemens, G. J., 1946 An analysis of the sex ratio among single and plural births in the total, the "white" and the "colored" U. S. populations. Am. J. Phys. Anthrop. N.S. **4**: 491–501.

Weinberg, W., 1902 Beiträge zur Physiologie und Pathologie der Mehrlingsgeburten beim Menschen. Pflüger's Arch. f. d. gesamt. Physiol. **88**: 346–430.

THE EFFECT OF X-RAYS UPON MUTATION OF THE GENE *A* IN MAIZE*

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THE experimental evidence indicating that gene mutation is induced by X-rays comes almost wholly from experiments on the general mutation rate; that is, from experiments in which the total frequency of detectable mutations at all loci is compared in treated and untreated cultures. "Gene mutations" as experimentally identified in such studies, include all variations inherited as if due to a change in a single gene. If it may be assumed that such variations can arise only from the transformation of a gene to an alternative form, these experiments prove the effect of the treatment upon some process of genic evolution.

It has long been evident that this assumption is not valid. Various extra-genic alterations may produce effects which are distinguishable only with great difficulty, if at all, from the effects expected from qualitative change in the hereditary unit. These include alterations involving loss, reduplication, and rearrangement of unaltered genes. To avoid ambiguity, the non-committal term "point mutation" will therefore be used to designate apparent gene mutations as experimentally identified, and the term "gene mutation" will be used only to designate the hypothetical conversion of a gene to an allelic form.

The inference that gene mutation is induced by X-rays is founded upon the assumption that some among the induced point mutations are in fact due to intragenic change, although others may be shown to be due to extra-genic alterations simulating gene mutation. But there are no general criteria by which the observed mutations occurring at miscellaneous loci may be individually identified as intra-genic or extra-genic. Consequently it is not possible to determine the effect of X-ray treatment upon gene mutation by experimental modification of the general mutation rate.

The alternative is to study the mutational behavior of selected genes especially suited to the purpose, both in spontaneous mutation and in mutation induced by various treatments, in the hope of developing for these genes a sounder basis for interpretation. For this purpose the loci *A* and *R* in maize provide favorable material, for reasons which have been stated in an earlier paper (STADLER 1941).

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The present study is concerned with the nature of X-ray-induced mutations at the *A* locus. A parallel study of ultraviolet-induced mutations at the *A* locus will be published shortly. These studies were made several years ago and the summarized results have been published in abstract form (STADLER and ROMAN 1943), but various delays incidental to the war have prevented the completion and fuller publication of the work. Meanwhile, certain other studies concerned with the general problem have been reported (STADLER and FOGEL 1943, 1945; STADLER 1944, 1946, FOGEL 1946a, b; LAUGHNAN 1946).

EXPERIMENTAL MATERIALS AND METHODS

The gene *A*, in the presence of appropriate complementary genes, affects the pigmentation of various tissues of the maize plant. In the presence of A_2, *B*, *Pl*, *C*, *R*, *Pr*, and *P*, the phenotypic effect of *A* may be represented as follows:

GENOTYPE	ALEURONE COLOR	PLANT COLOR	PERICARP COLOR
A A, *A a*	Purple	Purple	Red
a a	Colorless	Brown	Brown

The purple color of the aleurone and plant tissues is due to an anthocyanin pigment, while the red color of the pericarp is due to an unknown pigment, not an anthocyanin. Various additional alleles of *A* have been reported, including some with effects apparently intermediate between those of *A* and *a* (EMERSON and ANDERSON 1932; RHOADES 1941).

The populations from which the mutants here described were selected were F_1 progenies produced by the cross $aa \times AA$, the pollen of the male parent being X-rayed just before pollination. Plants showing loss of the *A* effect, whether by mutation, deficiency, or other types of genetic alteration, will be referred to as "*A* losses." These are readily identified in F_1, *A* loss in the endosperm resulting in a seed with colorless aleurone, *A* loss in the embryo in a seedling devoid of anthocyanin. Endosperm and embryo effects are produced independently in maize pollen irradiated at maturity, since the second microspore division occurs several days before the pollen is mature. Spontaneous mutations of *A* to *a*, occurring previous to gene reduplication for this division, would result in *A* loss in both endosperm and embryo. As determined from the frequency of mutation affecting endosperm and embryo together, the frequency of spontaneous mutation of *A* is low (STADLER 1941 and unpubl.), and no spontaneous mutations would be expected in a population equal to the total number of plants examined in this study.

With X-ray treatment of mature pollen, loss of the *A* effect is not uncommon, its frequency varying with the X-ray dose. *A* losses in the endosperm cannot be tested to discriminate between mutation and deficiency. The anthocyanin-free seedlings of the F_1, which include all *A* losses in the embryo, may include also maternal haploids or diploids resulting from development of the embryo without fertilization. The plants may be grown for further study, to distinguish these classes, and such alterations as are not lethal to the gametophyte may be carried on to subsequent generations for more critical analysis.

Haploids which survive the seedling stage are readily recognizable by their distinctive growth habit, reduced cell-size, and almost complete pollen abor-

tion, as well as by their wholly maternal phenotype. Maternal diploids would be recognizable only by their maternal phenotype. They would presumably be normal in development, and would be distinguishable from self-contaminations only by their failure to show the maternal phenotype in the endosperm of the seed from which the affected plant was grown. In cultures so marked as to permit identification of both maternal haploids and maternal diploids, more than 30 maternal haploids, but no maternal diploids, were found. The frequency of maternal haploids varies rather widely in different stocks used as female parent, and is not materially affected by X-ray treatment of the pollen parent, except for the slight increase in relative frequency expected from the elimination of a portion of the diploid progeny through lethal effects of the treatment.

The great majority of the *A* losses found were distinctly defective plants, among which those which survived to flowering had at least 50 percent of the pollen aborted. Among the remainder, with approximately normal plant development, similar segregation of aborted pollen is found in almost all cases. In a few of these plants the segregating defective pollen was not aborted, but was distinctly subnormal in size and in starch content at maturity. Many of these plants show segregation for two or more factors producing defective pollen, and in some of these there is segregation for both aborted and subnormal pollen. Various grades of defective pollen development are recognizable in iodine-stained pollen specimens, and specific grades are associated with specific deficiencies. These range form aborted pollen containing little or no starch, characteristic of most known deficiencies, to types only slightly subnormal in size and starch content. In a few cases the pollen is wholly normal in appearance but fails to accomplish fertilization in competition with normal pollen. McCLINTOCK (1944) has described minute terminal deficiencies in chromosome 9 with pollen visibly normal and capable of normal functioning in competition with non-deficient pollen. The occurence of segregating defective pollen serves as a rather sensitive detector of segmental deficiency, but deficiencies without detectable effect upon the pollen may occur.

The purpose of the experiment was to select from among the *A* losses induced by X-ray treatment those least subject to the suspicion of deficiency, in order to determine whether these were in fact experimentally indistinguishable from gene mutation of *A*. If *A* losses free from pollen effect occur, a certain proportion of these may be missed in the F_1 plants because of the coincidental occurrence of alterations at other loci resulting in defective pollen segregation. The proportion of such cases would increase with increasing dosage, and would be roughly equal to the proportion of pollen-segregating plants in the entire progeny. For this reason relatively low X-ray doses (usually 400 r) were used at first, but when it became evident that a very large sample of *A* losses would be needed, doses of 1000 r were used. Among a total of 126 *A* losses found in these progenies, 76 failed to survive to flowering or were so defective as to give no pollen specimen, 49 showed segregation for aborted pollen, or for aborted and subnormal pollen, and one showed segregation for subnormal pollen only. None was a normal plant free from defective pollen segregation. Since the pro-

portion of *A* losses free from gametophytic effect was obviously extremely low, various progenies produced for other purposes were used as a supplementary source of *A* losses. These were mostly from doses of 1000 r, though some with higher doses were included. They included progenies not so marked as to permit regular identification of haploids, and in some cases the genotype was such as to give anthocyanin-free seedlings by *R* loss as well as *A* loss. Among the anthocyanin-free plants which did not survive the seedling stage the *R* losses and in some cases the haploids could not be distinguished from the *A* losses. The inclusion of this supplementary material made it impossible to determine accurately the total number of *A* losses observed, but it did result in the finding of two *A* losses with no visible effect upon the pollen. A total of 543 anthocyanin-free seedlings was observed in the course of the experiment. On the basis of the relative frequency of haploids and *R* losses in cultures properly marked for their identification, it is estimated that about 415 of these plants were *A* losses.

THE MUTANTS

The genetic alterations responsible for the two *A* loss plants with normal pollen were designated respectively *a-X1* and *a-X2*, and were extracted for further study. In addition there was included for comparison the genetic alteration extracted from one of the *A* losses with segregating subnormal pollen. This was designated *a-X3*.

Alteration *a-X1* occurred in a plant of normal vigor in an F_1 progeny of the cross *a* A_2 R^g *C b pl-y pr wx p*×*A* A_2 r^{ch} *C b pl-y Pr Wx p*, in which the pollen was irradiated (dose 1000 r) just before pollination. The genotype was such as to give green plants by either *A* loss or r^{ch} loss. The ear, pollinated by a^p *Dt Dt*, yielded a full seed set, showing that the alteration was haplo-viable and transmissible through female germ cells. All of the seeds were pale, showing that the case was due to *A* loss rather than r^{ch} loss. Somewhat less than half of these seeds showed one or more dots, and the remainder were dotless. The plants grown from these seeds were crossed on and by an *a*-tester (*a* A_2 *C R*). In crosses on the *a* tester stock all of the plants from dotted seeds gave approximately equal transmission of pale and colorless, while all but one of the plants from dotless seeds showed lowered transmission of the colorless seed type. Subsequent tests confirmed this indication, showing that the *a* "allele" derived from the treated parent was distinguished from the standard *a* allele by failure to respond to *Dt* (in any dosage) and by consistently lowered transmission through male germ cells.

Alteration *a-X2* was found in F_1 of the cross *a* A_2 *C* R^g *b pl p*×*A* A_2 R^r *C b Pl p*, with treatment of the parental pollen at a dose of about 900 r just before pollination. The treatment was applied in the field with a mobile X-ray unit not calibrated in r units. The dose is estimated from the relative frequency of genetic effects commonly produced by this treatment, in comparison to the frequency of similar effects produced by treatments at measured doses. The plant was normal in vigor and produced two ears, both with approximately a full set of seed. Pollinations similar to those described for *a-X1* gave similar results, except for a greater reduction in transmission through male germ cells

and an indication in some ears of possible reduced transmission through female germ cells.

Alteration *a-X3* occurred in the F_1 of the cross *a* A_2 *C-Wx* r^r *j lg*×*A* A_2 *c-wx* R^g *pr su y*, in which both the parental ear and the parental tassel were X-rayed just before pollination. This treatment also was applied in the field. The dose applied to the tassel was approximately 1200 r. The original F_1 plant was normal in development, but about 50 percent of its pollen was clearly subnormal in size and starch content. A similar subnormal pollen type, associated with an *R* deficiency (*DfX-1*) has been illustrated and described in some detail in an earlier paper (STADLER 1933). The ear of this plant did not produce a full seed set, but in parts of the ear there seemed to be definitely more than 50 percent seed set. Cultures grown from the seed in these sections yielded a few plants with pollen segregation similar to that observed in the F_1 plant, and these were crossed with various testers to provide material for further study. Pollinations similar to those described for *a-X1* showed that the subnormal pollen segregation is regularly associated with *a-X3*, and that there is no transmission of the alteration through male germ cells. The subnormal pollen is slightly less defective than in *Df X-1*, but is always clearly detectable. Transmission through female germ cells also is distinctly reduced. Its frequency is readily shown in pollinations of the type *A*/*a-X3*×a^p, in which the seeds heterozygous for *a-X3* are pale, while their normal sibs are fully colored. The pale seeds in this pollination are usually less than one fourth as numerous as the colorless seeds, and in general are slightly smaller. The difference in seed size is slight, and would not be detectable in the shelled seed, because of difference in seed size due to position of the seed on the ear. The reality of the difference may be demonstrated objectively in ears of the type *A*/*a-X3*×*A*, in which the two classes are indistinguishable in color-phenotype. Small seeds, carefully selected by comparison of seed size in neighboring seeds, give with few exceptions plants showing subnormal pollen segregation. The plants grown from the small seeds are not visibly inferior to their sibs, either in seedling growth or in development at later stages.

All three alterations occurred in cultures lacking the complementary factors, *B*, *Pl*, and *P*. In subsequent extractions it was found, for all three alterations, that plants of genotype *a*/*a-X B Pl* are brown, like *a*/*a B Pl*, and plants of genotype *a*/*a-X P* have brown pericarp like *a*/*a P*. Several objective tests were made in an attempt to distinguish *a*/*a-X3* from *a*/*a* sibs on the basis of plant color phenotype at various stages of development, but these attempts were wholly unsuccessful.

GAMETOPHYTE VIABILITY

In crosses of the type *A*/*a-X* ×a^p, the production of pale seeds demonstrably *a-X*/a^p in genotype proves that the *a-X* alteration is viable in the haploid female gametophyte, for these seeds can be produced only by the normal functioning of the *a-X* gametophyte. Similarly, the production of a^p/*a-X* seeds in crosses of the type a^p×*a*/*a-X* proves that the alteration is viable in the male gametophyte. On the basis of these tests it is clear that *a-X1* and *a-X2* are viable in both male and female gametophytes, and that *a-X3* is viable at least in

female gametophytes. The failure of male transmission of *a-X3* does not necessarily mean that pollen bearing this alteration is inviable, since it could result from the slower pollen tube growth of this type in competition with pollen bearing the standard allele *a*, as well as from failure of the pollen to germinate.

The pollinations made to determine haplo-viability show also the relative frequency of fertilization accomplished by *a-X* gametophytes versus gametophytes bearing a standard *A* or a^p allele. Differences in female transmission between two contrasted types presumably result only from differences in the proportion effectively fertilized or differences in survival of the heterozygotes produced. Differences in male transmission may result from these causes also, but in addition the factor of competition in pollen-tube growth may cause large inequalities in the number of ovules which may be fertilized by the two types of sperms. Trials of male transmission frequency are complicated by the fact that there are many unidentified pollen tube growth factors in maize, so that 1:1 transmission through male germ cells is not necessarily to be expected in the case of two alleles which are themselves without effect upon gametophyte functioning. A significant deviation from equality may be the result of other factors linked to those under study. For more critical evidence of the effect, it is desirable to make comparisons of male transmission between sibs secured from a cross of the type $A \times a\text{-}X/a$, the transmission tests being crosses of a number of the progeny plants, including $A/a\text{-}X$ and A/a individuals, upon an *a^{dl}-tester* (a^{dl} A_2 *C R Dt Dt*). The symbol a^{dl} represents an allele similar to *a* except for failure to respond to *Dt*. Colorless seeds in the crosses by A/a are dotted; those in crosses by $A/a\text{-}X$ are dotless. The relative frequency of colored and colorless seeds in the crosses by A/a plants establishes a norm of transmission of the *A* chromosome for comparison with that shown in crosses by the $A/a\text{-}X$ plants.

Trials of this type were made in various extracted stocks of both *a-X1* and *a-X2*, and trials of female transmission in several extracted stocks of each of the three alterations. Alteration *a-X1* regularly shows reduced male transmission, but the extent of reduction varies in different cultures. For example, in tests of $a\text{-}X1/a^p$ plants, in comparison with a/a^p sibs, seven $a\text{-}X1/a^p$ plants of one culture yielded a total of 803 colorless and 1390 pale seeds (58 percent of normal transmission), while in a closely related culture tested at the same time five plants tested yielded 317 colorless and 742 pale (43 percent of normal transmission). The individual plants did not differ significantly from the averages stated, and the a/a^p sibs in both cultures gave normal transmission. Occasional plants heterozygous for *a-X1* have been found to give less than 25 percent normal transmission, but ordinarily male transmission of this alteration is not far from 50 percent of normal. Female transmission is usually equal to normal, with occasional exceptions showing slightly lowered transmission.

With *a-X2*, male transmission is commonly about 25 percent of normal, and female transmission about 65 percent of normal, again with occasional rather wide variations. One culture, in which seven plants of $A/a\text{-}X2$ and six plants of A/a were all tested for both male and female transmission, gave a total frequency of 1334 *A* and 398 *a-X2* in male transmission (30 percent normal) and

931 *A* and 661 *a-X2* in female transmission (71 percent normal). Differences between individual plants were slight and transmission was normal in the *A/a* sibs. In other cultures male transmission has frequently been as low as 15 percent and occasionally as low as 5 percent. For example, in one culture in which three a^p/a-*X2* plants were tested rather extensively for male transmission, the total frequency was 1588 a^p and 72 *a-X2* (4.5 percent normal).

With *a-X3*, male transmission has never been found, and female transmission is always low, ordinarily about 15–35 percent. Individual ears with no transmission of the alteration are not rare, but usually at least a few *a-X3* seeds are produced, and occasionally female transmission approaches 50 percent.

VIABILITY OF HOMOZYGOTES AND COMPOUNDS

When *A/a-X1* or a^p/a-*X1* plants are selfed or intercrossed, the homozygote *a-X1/a-X1* should be recognizable by absence of anthocyanin in the aleurone and plant tissues, and should occur with an average frequency of about 17 percent (assuming normal female transmission and 50 percent male transmission of *a-X1*). Selfed or intercrossed ears of these genotypes yield no colorless seeds at all. The absence of colorless seeds cannot be accounted for by the assumption that the phenotype of *A-X1/a-X1* is other than as expected, for all of the seeds from *A/a-X1* selfs are full-colored, and all of the seeds from a^p/a-*X1* selfs are pale. The plants grown from these seeds show the corresponding plant color phenotype, and at least half of these plants are found to be heterozygous for *a-X1*. In many of these pollinations, the same pollen sample used in selfing was used also in outcrosses on an *a* tester, to establish the level of male transmission of *a-X1*, and sibs of the plants selfed were pollinated by an *a* tester to establish female transmission of *a-X1* in the cultures used. Male and female transmission tests of *a-X1* in the outcrosses gave results in agreement with the tests described in the preceding section. Assuming random fertilisation, the absense of colorless seeds thus shows that the homozygote is inviable. There was no class of aborted or defective seeds, or of empty pericarps, which could be considered to represent the ovules in which the combination occurred.

With *a-X2* the proportion of homozygotes expected in selfs and intercrosses is lower, due to the lower male and female transmission of this alteration. With female transmission at 65 percent and male transmission at 25 percent of normal, the expected average frequency of homozygotes would be about 8 percent. A large number of selfs and intercrosses of *a-X2* heterozygotes, including several with simultaneous tests of male and female transmission, failed to yield a single colorless seed, and it is clear that in this case also the absence of colorless seeds is due to the inviability of the homozygote.

The viability of *a-X3* homozygotes cannot be tested in this manner, because of the lack of male transmission.

The viability of the compound *a-X1/a-X2* is readily tested by crosses of the type a^p/a-*X2*$\times a^p/a$-*X1*. The expected frequency of colorless seeds representing the compound is about 13 percent, or for the reciprocal cross about 10 percent. Numerous crosses of these types were made, and all of the seeds produced were pale, except for one colorless seed. The plant grown from this seed proved

to be heterozygous for a dottable *a*. The seed thus does not represent the compound, and presumable was the result of pollen contamination.

Viability of the compounds *a-X1/a-X3* and *a-X2/a-X3* may be tested similarly, but because of the low and variable female transmission of *a-X3* the tests must be made on a fairly large number of ears of the *a-X3* heterozygote. Crosses of the type $a^p/a\text{-}X3 \times a^p/a\text{-}X1$ were made on 11 ears, which produced a total of 985 seeds, all pale. With average transmission of the *a-X* alterations concerned, and with normal viability of the compound, the expected frequency of colorless seeds would be 55 (5.6 percent). Similarly, 13 ears of the cross $a^p/a\text{-}X3 \times a^p/a\text{-}X2$ yielded a total of 1130 seeds, all of which were pale except two colorless seeds which proved to be due to pollen contamination. With average transmission rates the expected frequency of colorless seeds would be 37 (3.3 percent). These crosses indicate beyond reasonable doubt that the compounds in all combinations, like the homozygotes, are zygotically lethal.[1]

EFFECT ON CROSSING OVER

Short intercalary deficiencies in maize sometimes reduce crossing over much more than would be expected from the length of the deficient segment. For example (STADLER 1935) *Df 5-1*, a deficiency of a short intercalary segment of the longer arm of chromosome 5, includes the locus V_3, and not the neighboring loci *Bt*, to the left, and *Bv*, to the right. Its genetic length is therefore less than 3.8 crossover units, the map distance from *Bt* to *Bv*, The deficiency is haplo-viable in the female gemetophyte, and in heterozygous plants it is cytologically detectable at pachytene by the occurrence of a short "buckle" in chromosome 5, usually located on the longer arm near the centromere. In plants heterozygous for *Df 5-1*, crossing over in the region between *Bm* and *Bv* (the region including the deficiency) is almost completely inhibited, and crossing over in the adjacent region between *Bv* and *Pr* is greatly reduced. The total reduction in crossing over is considerably greater than the map length of the deficiency.

Presumably, the reason for the reduction in crossing over outside of the deficient region is the strong tendency toward non-homologous pairing in the chromosomes of maize. MCCLINTOCK (1933) has shown, in plants heterozygous for various chromosomal derangements, the frequent occurrence of close pachytene pairing of non-homologous regions. In plants heterozygous for *Df 5-1*, the buckle observed in the chromosome 5 pair at pachytene is formed by a segment of the non-deficient homolog equal in length to the segment missing from the deficient homolog. If pairing were always between homologous regions, the position of the buckle would be constant and would mark the location of the deficiency. In fact, however (STADLER 1935), the position of the buckle varies widely, and in extreme instances it may occur on the distal half of the longer arm, or in the proximal region of the shorter arm. When the position of the buckle is not identical with the locus of the deficiency, the chromosome re-

[1] Note added in proof: RHOADES (Maize News Letter, Mar. 1948), using *a-X2* in combination with a gametophyte factor *ga* distal to *et*, finds no production of homozygous *a-X2* seeds on selfed ears of the heterozygote *ga/a-X2*. Here the ratio expected is increased to about 30 percent, and the absence of visible sterility on the ear suggests that selective fertilization may be involved.

gions paired between these two points must be non-homologous. In cases in which the buckle is found on the shorter arm, the non-homology of the paired region is cytologically evident, for the centromeres of the two chromosomes are separated by a distance approximately equal to the length of the buckled segment.

If non-homologous pairing is a major cause of the reduction in crossing over, it may be expected that pronounced reduction in crossing over may usually be found with short intercalary deficiencies in maize. The cytological observations described above are typical of the observed behavior of short intercalary deficiencies. Deficiencies too short to cause the formation of a visible buckle, though they would not permit its cytological demonstration, could result in non-homologous pairing which might extend over a considerable length of chromosome and thus might lead to distinct reduction in crossing over.

The effect of the *a-X* alterations upon crossing over can be determined only with rather distant or otherwise unfavorable marker genes. The only known marker distal to *A* is *et*, an X-ray-induced mutant located about 12 units from *A* (STADLER 1940). The phenotypic effect of *et* is shown by both endosperm and seedling, the endosperm surface being characteristically scarred (etched), and the seedling being virescent. The endosperm effect is usually distinct enough for positive separation, though it may sometimes be confused with scarred endosperm resulting from the action of other genes or occurring sporadically with no simple genetic basis. In some matings the endosperm effect is very extreme, and many of the etched seeds are small and inviable. The virescent character of the seedling is always clear and readily separable, except at unusually high temperatures. Cultures which give a clear etched endosperm separation always give virescent seedlings from all etched seeds, and in ears with endosperm separation doubtful, the seedling separation is useful as a check. The alteration is readily transmitted through both male and female germ cells, though sometimes in less than normal ratio.

The nearest useful locus proximal to *A* is lg_2, about 34 crossover units distant. The gene *na* is probably a few units closer to *A* but is not well suited to seedling detection. The effect of lg_2 is clear in the seedling stage, permitting positive classification in the rather large numbers needed for the comparison.

The effect of the *a-X* alterations upon crossing over in the regions *Et-A* and *A-*Lg_2 was determined from sib plants produced in crosses of the type *Et a-X* $Lg_2/Et\ a\ Lg_2 \times et\ A\ lg_2$. This cross yields plants of two types, *Et a-X* $Lg_2/et\ A\ lg_2$ and *Et a* $Lg_2/et\ A\ lg_2$. Crossover frequency was determined in plants of both types, by pollinating them by *et a* lg_2. The results are shown in table 1. The relationship of the plants tested is indicated by the culture numbers. For example, the effect of *a-X1* was tested in two cultures, 49:763 (including two *a-X1/A* plants and three *a/A* plants tested) and 51:14 (including three *a-X1/A* and three *a/A* plants tested). The cultures 49:763 and 51:14 were progenies of two different crosses of the type *Et a-X* $Lg_2/Et\ a\ lg_2 \times et\ A\ Lg_2$.

With *a-X1* there is no consistent indication of crossover modification, although the average frequency of crossing over in the *Et-A* interval is slightly lower than in the normal sibs tested.

With *a-X2*, there is a pronounced and consistent reduction in crossover fre-

TABLE 1

Effect of a-X1, a-X2, and a-X3 on crossing over. Crossover frequency in female gametes in backcrosses.

F_1 GENOTYPE	NON-CROSSOVERS		CROSSOVERS						CROSSOVER %	
			REG. 1		REG. 2		REG. 1, 2		REG. 1	REG. 2
					a-X1					
et A lg/Et a-X1 Lg										
49:763-3	44	57	7	8	22	26	2	0	10.2	30.1
49:763-12	106	85	11	16	36	52	4	2	10.6	30.1
51:14-6	98	110	10	14	55	48	6	5	10.1	33.0
51:14-13	88	70	19	20	41	47	2	2	14.9	31.8
51:14-19	69	67	13	13	21	14	2	9	17.8	22.1
Total	405	389	60	71	175	187	16	18	12.5	30.0
et A lg/Et a Lg										
49:763-4	80	81	25	24	26	45	2	1	18.3	26.1
49:763-9	81	82	19	16	36	39	3	2	14.4	28.8
49:763-10	42	61	13	10	28	26	2	6	16.5	33.0
51:14-5	72	70	17	16	35	29	0	4	15.2	28.0
51:14-9	93	85	16	16	36	58	4	8	13.9	33.5
51:14-20	82	62	6	18	28	31	4	3	13.3	28.2
Total	450	441	96	100	189	228	15	24	15.2	29.6
					a-X2					
et A lg/Et a-X2 Lg										
49:774-7	86	62	0	5	26	28	0	3	3.8	27.1
49:774-8	43	60	2	7	12	9	0	0	6.8	15.8
51:15-2	63	110	2	9	12	25	1	1	5.8	17.5
51:15-4	21	19	0	3	3	6	0	0	5.8	17.3
51:15-11	62	82	1	4	13	35	1	1	3.5	25.1
51:15-13	48	64	1	8	16	9	1	3	8.7	19.3
51:15-14	57	90	2	1	14	25	0	0	1.6	20.6
51:15-17	68	97	4	9	13	34	0	1	6.2	21.2
51:15-18	45	92	2	5	10	18	1	0	4.6	16.8
Total	493	676	14	51	119	189	4	9	5.0	20.6
et A lg/Et a Lg										
49:774-9	16	54	3	10	15	31	1	0	10.8	36.2
49:774-11	28	67	7	11	13	28	1	2	13.4	28.0
49:774-14	40	42	7	15	19	32	1	4	16.9	35.0
51:15-3	55	100	7	19	35	52	2	1	10.7	33.2
51:15-15	60	113	16	12	31	77	2	6	11.4	36.6
51:15-19	55	120	10	19	25	65	1	2	10.8	31.3
Total	254	496	50	86	138	285	8	15	11.9	33.5
					a-X3					
et A lg/Et a-X3 Lg										
49:751.1-1	93	7	0	2	37	4	0	0	1.4	28.7
49:751.1-3	122	25	0	1	45	12	0	0	0.5	27.8
Total	215	32	0	3	82	16	0	0	0.9	28.2
et A lg/Et a Lg										
49:751.2-1	110	109	20	17	52	53	2	0	10.7	29.5
49:751.2-3	19	101	9	6	44	20	0	14	13.6	36.6
49:751.2-7	41	51	6	8	30	32	1	4	11.0	38.7
49:751.2-9	85	77	17	13	27	42	7	2	14.4	28.9
Total	255	338	52	44	153	147	10	20	12.4	32.4

quency, both in the *Et-A* interval, to the left of the alteration, and in the *A-Lg*$_2$ interval, to the right. There is considerable variation in the extent of reduction of crossing over in different plants, but in each of the nine *a-X2/A* plants tested, for both of the segments covered, the crossover frequency is lower than in any of the six *a/A* plants tested.

With *a-X3*, only two plants heterozygous for the alteration are available in the test for crossover effects in the entire *Et-Lg*$_2$ region, since in the tests made in the later series it was necessary to use cultures not marked at the *Lg*$_2$ locus.

TABLE 2

Effect of a-X3 on crossing over (Et-A segment). Crossover frequency in female gametes in backcrosses.

F_1 GENOTYPE	NON-CROSSOVERS		CROSSOVERS		CROSSOVER PERCENT
	et A	*Et a(−X3)*	*et a(−X3)*	*et A*	
et A/Et a-X3					
49:751.1–1	130	11	0	2	1.4
49:751.1–3	167	37	0	1	0.5
51:11.1–1	30	24	0	3	5.3
51:11.1–10	91	64	1	6	4.3
51:11.2–8	64	63	0	10	7.3
51:12.1–12	68	19	0	4	4.4
51:12.1–14	71	10	0	8	9.0
Total	621	228	1	34	4.0
et A/Et a					
49:751.2–1	162	162	22	17	10.8
49:751.2–3	63	121	9	20	13.6
49:751.2–7	71	83	7	12	11.0
49:751.2–9	112	119	24	15	14.4
51:11.1–11	95	127	14	8	9.0
51:11.2–6	71	141	9	23	13.1
51:11.2–22	120	133	12	18	10.6
51:12.1–11	66	136	23	17	16.5
51:12.1–13	86	176	10	16	9.0
Total	846	1,198	130	146	11.9

The data on crossing over in the *Et-A* region in these tests will be given presently. The data for the two plants marked at all three loci show pronounced reduction of crossing over in the *Et-A* region, but only slight and insignificant reduction in the *A-Lg*$_2$ region.

Additional data for *a-X3*, on crossing over in the *Et-A* region, are given in table 2. The endosperm classification was checked by seedling classification in all cases, and seeds which failed to germinate are omitted. The data are therefore comparable with those for the *Et-A* region in table 1. Data on the *a-X3* effect from table 1 are included in table 2 for comparison.

The results show a pronounced reduction in crossing over in plants hetero-

zygous for *a-X3*, similar to that observed with *a-X2*, and like the latter varyng rather widely in different F_1 plants tested.

It should be noted that there are large inequalities in the frequency of corresponding classes in these backcross progenies. This is evident not only in the progenies of *A/a X* plants, in which inequality is expected from the lowered female transmission of the various *a-X* alterations, but also in the progenies of *A/a* plants, in which this factor would not enter. The marker gene *et* shows distinctly reduced viability in the families used in testing the crossover effect of *a-X2* and *a-X3*, in which the progenies of *A/a* plants used as controls show *Et:et* ratios of 1.00:0.51 and 1.00:0.73 respectively. The reduced frequency of

TABLE 3

Effect of a-X1, a-X2, and a-X3 on crossing over (ET-A segment). Data from totalled seed counts. (Crossover frequency in female gametes in backcrosses.)

F_1 GENOTYPE	NUMBER OF CULTURES	NON-CROSSOVERS		CROSSOVERS		CROSSOVER percent	*Et:et* RATIO	*A:a(-X)* RATIO
		et A	*Et a(-X)*	*et a(-X)*	*Et A*		1.00:	1.00:
For *a-X1*								
et A/Et a-X1	5	598	597	78	90	12.3	0.98	0.98
et A/Et a	6	676	683	114	132	15.3	0.97	0.99
For *a-X2*								
et A/Et a-X2	9	737	892	27	61	5.1	0.80	1.15
et A/Et a	6	484	798	61	108	11.7	0.60	1.45
For *a-X3*								
et A/Et a-X3	7	917	261	8	37	3.7	0.32	0.28
et A/Et a	9	1,036	1,261	166	153	12.2	0.85	0.83

et is due in part to lowered female transmission and in part to lowered germination of *et* seeds in some of the cultures. Both tendencies vary widely in different families; note for example that the *Et:et* ratio in progenies of *A/a* plants used as controls for *a-X1* effects is 1.00:0.95.

Since *Et* and *A* are both identified by seed as well as seedling effects, inequalities due to differences in germination may be excluded from data on the *Et-A* region by using the seed counts rather than the seedling counts. All of the data relating to *Et-A* crossing over in tables 1 and 2 were recalculated in terms of the total population of seeds produced. Since the seedling results corrected certain errors of seed classification for *et*, the corrected figures were used, and in the classes affected the same proportionate correction was applied to the seeds which failed to germinate. This recalculation made slight changes in the crossover frequencies observed in individual cultures, but made no material change in the indications of effects on crossing over. In the case of both *a-X2* and *a-X3*, in the data from total seed counts, crossover frequency was lower in every *A/a-X* progeny than in any of the control *A/a* progenies. The effect upon crossover frequency in the totalled data for each group is shown in table 3.

It is evident that marked inequalities of the *Et* and *et* classes remain in the families used for the *a-X2* and *a-X3* comparisons, with a reduction of female transmission of *et* to about 60 percent and 85 percent respectively in the cultures from the *et A/Et a* control plants. In the control cultures this does not distort the crossover frequency, since it affects the crossover and non-crossover classes equally. But in the cultures segregating also for an *a-X* allele of reduced viability, the effect upon these two classes is necessarily unequal. Is this factor, rather than an actual decrease in crossing over, the cause of the reduced frequency of crossover gametes observed in the *A/a-X* plants?

Assume, for example, a crossover frequency of 12 percent in the *et A/Et a-X2* plants, as in their control sibs. In an ear with a potential yield of 200 seeds, we expect 88 gametes of each of the non-crossover types and 12 of each of the crossover types. Assuming 60 percent survival for *et*, the frequency of the *et A* class is reduced from 88 to 53. The frequency of the *Et a-X2* class will be similarly reduced by lower female transmission of *a-X2*, but the extent of this reduction varies in different cultures. We may estimate the reduction in these plants directly by comparing the *a-X* frequency observed with that expected if *a-X* had no effect on viability. With 12 percent crossing over and with full survival of *a-X2*, these cultures would be expected to have a ratio for *A:a-X* of 1.00:1.47, the reduced frequency of *A* being due to the disproportionate elimination of *A* individuals by the action of *et*. Actually the ears had a ratio for *A:a* of 1.00:1.15, indicating survival of *a-X2* at about 78 percent of normal. The expected frequencies of the four classes (with no interaction of viability effects) would thus be

	et A	*Et a-X*	*et a-X*	*Et A*	TOTAL	CROSSOVERS	% OF CROSSOVERS
Gamete ratio	88	88	12	12	200	24	12.0
Survival rate	.60	.78	.47 (.60×.78)	1.00			
Expected frequency	53	69	6	12	140	18	12.9

The effect is thus to increase slightly the apparent frequency of crossing over. With lower survival of gametes, such as might occur with *a-X3*, the error would be increased materially, as shown below:

	et A	*Et a-X*	*et a-X*	*Et A*	TOTAL	CROSSOVERS	% OF CROSSOVERS
Gametes	88	88	12	12	200	24	12.0
Survival rate	.60	.25	.15	1.00			
Expected	53	22	2	12	89	14	15.7

It is clear that this error in estimating crossover frequency, when the viability factors are in repulsion-phase, must always be in the plus direction. The apparent increase in crossover frequency is due to an unbalanced reduction in survival of the various classes of gametes, and while both non-crossover classes are reduced in survival, only one of the two crossover classes is so affected.

The illustrations given assume no interaction of the viability factors. For example, in the first illustration, it is assumed that of the 12 gametes of geno-

type *et a-X2* expected, 40 percent would be eliminated by the action of *et.* and of the remainder, 22 percent would be eliminated by the action of *A-X2*, It would not be surprising if the survival of *et a-X2* gametes were less than the expected 47 percent, since the effect of both viability factors in the same individual might be greater than would be anticipated from their effects in different individuals. But even if the cumulative effect were much increased, it could not result in any large decrease in apparent crossovers compared with the control, for it could only affect individuals in the single class which provides only a minority of the observed crossovers. At its extreme, in the example given, with complete elimination of this class, it would give an apparent reduction of crossing over from 12 percent to 8.6 percent. In the second illustration, with lower survival, the maximum decrease possible would not be sufficient to balance the spurious increase in observed crossovers, and the results with complete elimination of the *et a-X* class would still indicate an increase in crossovers over the control. Obviously, the reduction of crossovers to less than half the control frequency, as found with both *a-X2* and *a-X3*, could not be due to these sources of error.

The experimental results (table 3) show further that the *et a-X* class was not eliminated; it provides 27 of the 88 crossovers observed with *a-X2* and 8 of the 45 with *a-X3*. Is this inequality of the crossover classes, and the observed inequality of the non-crossover classes, reasonably accounted for on the basis of viability differences involved?

Consider first the experimental data for *a-X2* (table 3). The survival ratio for *et* in the families used is 0.60, as indicated by the control cultures. With 60 percent survival of *et* in the *et A/Et a-X2* cultures, and with full survival of *a-X2*, these ears should have produced 1228 *Et a-X2* seeds (737/0.60) and 37 *et a-X2* seeds (61×0.60). Actually they produced 892 *Et a-X2* and 27 *et a-X2* seeds, indicating survival of about 73 percent in both classes. In other words, on the assumption of 60 percent survival for *et*, 73 percent survival for *a-X2*, and no interaction of viability effects, a gametic population of 2456 non-crossovers and 122 crossovers would have given the results observed. This represents an actual crossover percentage of 4.7, though among the surviving individuals crossovers would constitute 5.1 percent.

A similar calculation for the *et A/Et a-X3* cultures is shown below:

	et A	*Et a-X3*	*et a-X3*	*Et A*
Observed (table 3)	917	261	8	37
Expected with full survival of *a-X3*	917	1079	31	37
Indicated survival of *a-X3*		24%	26%	
Indicated gametic ratio	1079	1079	37	37

Here also the inequality of the crossover classes is well accounted for by the relative viability of the genotypes concerned, and the crossover frequency indicated, 3.3 percent, is a little lower than that shown by the percentage of crossovers among survivors.

Thus, in spite of the rather unfavorable marker genes necessarily used, the data show pronounced effects of *a-X2* and *a-X3* on the frequency of crossing over. The effect is unexpectedly large, *a-X2* heterozygotes showing a reduction

of about 20 crossover units in the *Et-Lg*$_2$ segment. In *a-X3* heterozygotes, which were adequately tested only in the *Et-A* segment, the reduction in frequency of crossing over in this region was even greater than that shown by *a-X2*.

CYTOLOGICAL OBSERVATIONS

The pronounced effect of *a-X2* and *a-X3* upon crossing over suggests an effect of these alterations on chromosome pairing in the region of the *A* locus. The additional effect of *a-X3* upon pollen development, and its sharply reduced female transmission, suggests further deficiency at this locus. Cytological examinations were made therefore of plants heterozygous for *a-X3*, in the pachytene stage of the microsporocyte.

The *A* locus is in the terminal one-fifth of the long arm of chromosome 3 (McCLINTOCK 1931). This region was carefully examined in two types of material. In the first of these, the normal chromosome 3 carried a distinctive knob, near the midpoint of the long arm, which facilitated identification of the chromosome. Repeated observations failed to show any consistent abnormality in the region concerned. However, the material was not well suited for the detection of small alterations since the knob itself, in heterozygous condition, was responsible for some looseness and the frequent occurrence of buckles, usually in the vicinity of the knob but sometimes also in adjacent regions. More critical material was obtained from plants heterozygous for *a-X3* but lacking the knob. Several pachytene configurations were examined in which chromosome 3 was positively identified. In these there was no suggestion of a deficiency buckle or an inversion loop in the region of the *A* locus; the strands were as closely paired in this region as in other regions of the chromosomes.

The examinations made show that no reciprocal translocation is present, and no deficiency, insertion, or inversion long enough to interfere with visually normal pairing of the affected chromosome with its normal homolog. However, they do not exclude the possibility of a minute alteration that might be detectable only if located in a region most favorable for cytological study. All three *a-X* alterations, if due to deficiency, must be intercalary since the gene *Et*, located distal to *A* and present in the stocks originally treated, was not lost in any of the alterations. It is doubtful that losses as small as the minute terminal deficiences of chromosome 9, recently described by McCLINTOCK (1944), would be cytologically detectable if they occupied an intercalary position.

EVIDENCE FROM THE USE OF AN UNSTABLE DUPLICATION

The unstable duplication used in the experiments now to be described was secured indirectly from a chromosomal aberration found in a progeny from ultraviolet-treated pollen. A stock carrying A^b was irradiated and crossed on an *a* tester stock, in order to secure *A* losses from ultraviolet treatment for study in comparison with those from X-ray treatment. One of the plants of this progeny was a sectorial chimera, in which most of the plant showed the *a* phenotype but a sector of considerable size was of A^b phenotype. Both sectors extended into the tassel, and pollinations made from A^b anthers upon *a* tester

ears showed some transmission of A^b (39 colored seeds+7 colored-colorless mosaics+260 colorless).

The colored seeds yielded colored plants, typical of A^b. A few of these were found to show small sectors of *a* tissue. The colored-colorless mosaic seeds regularly gave plants variegated for A^b and *a* tissue, suggestive of the ring-chromosome variegation types described by McCLINTOCK (1938). A typical plant of this sort is shown in fig. 1, A. Cytological examination of these plants showed at pachytene a short supernumerary fragment in addition to the normal complement of ten pairs of chromosomes. This is designated *Dp 3a*, and will be referred to in this paper simply as *Dp*. The fragment was never seen as an open ring though it may have been a collapsed ring. Cytological study of the aberration was undertaken by DR. HELEN V. CROUSE, and will be reported separately.

This unstable duplication covering the *A* locus provides an opportunity for further study of the *a-X* alterations. With the addition of this duplication, the homozygotes and compounds of the *a-X* alterations may be shielded from the zygotic lethal effect, and, if these genotypes are viable in any tissue, the subsequent loss or diminution of the duplication fragment might make it possible to observe their effects.

Crosses were made with all three *a-X* alterations, according to the scheme illustrated below in the case of *a-X1:*

(1) $a\text{-}X1/a^p \times a\ a,\ Dp.$

Most of the seeds produced by this pollination are pale or colorless. There are in addition a considerable number of seeds showing full color due to A^b, but all of these are endosperm mosaics. In about half of these mosaics the sector lacking full color is pale; in the remainder it is colorless. The latter class represents the genotype *a-X1/a, Dp*. The plants grown from these seeds show a sectorial phenotype similar to *a a, Dp* (fig. 1, A). A seed showing a typical endosperm mosaic of this class is shown in fig. 3, A.

(2) $a\text{-}X1/a^p \times a\text{-}X1/a,\ Dp.$

This cross similarly yields pale and colorless seeds together with colored seeds showing endosperm mosiacs. Among the latter a new type appears, in which the region lacking color, instead of being healthy endosperm tissue distinguished only by pigmentation of the aleurone layer, is shriveled, degenerate tissue. An example is shown in fig. 3, B.

Seeds of this type are *a-X1/a-X1, Dp*. Plants grown from these seeds are variegated, but are of a type very different from their *a X1/a* (or a^p), *Dp* sibs which resemble the plant pictured in fig. 1, A. A typical plant of *a-X1/a-X1, Dp* is pictured in fig. 1, B. The sectors are all relatively small, and in general they are marked by absence of chlorophyll rather than absence of anthocyanin. The plants are distinctly defective in development, but they usually survive to maturity, and produce both tassels and ears. Flowering is delayed, and seed yield is small.

(3) $a\text{-}X1/a^p \times a\text{-}X1/a\text{-}X1,\ Dp.$

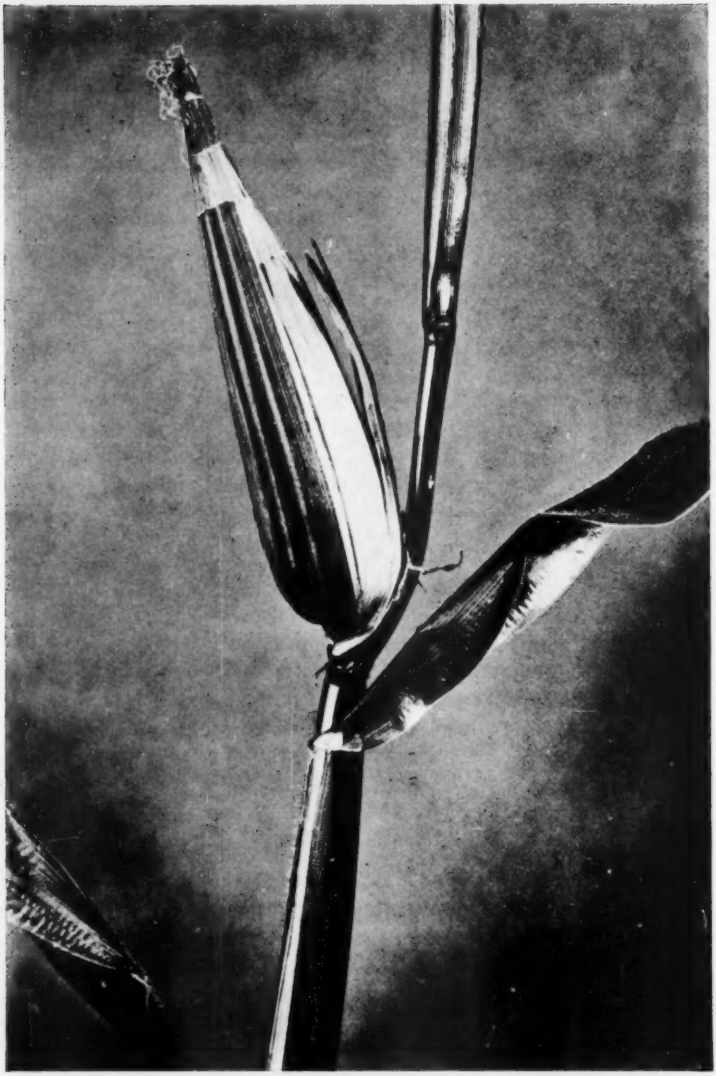
Crosses of this type are used to produce the *a-X/a-X, Dp* types in quantity, since all of the seeds produced are colored seed mosaics. The mosaics not showing degenerate sectors are mosaic for pale, not colorless.

Compounds are produced by appropriate substitution of the various *a-X* alterations in crosses (2) and (3). The chief difficulty in extracting the types desired comes from the low transmission of *a-X3*, and to a lesser extent of *a-X2*, in the heterozygotes lacking the duplication. Both alterations are readily transmitted through pollen from plants carrying the duplication. Successful extraction of *a-X2* or *a-X3* in cross (2) thus makes the production of their compounds with *a-X1* a simple matter, but the extraction of the homozygotes requires further use in cross (3) of the non-duplication stocks of low transmission. It was ultimately possible to extract all three homozygotes and all three compounds. The types *a-X3/a-X3, Dp* and *a-X2/a-X3, Dp* were first secured in crosses using *Dp* stocks as both male and female parents, a procedure which is undesirable for some purposes because of the possible inclusion in the progeny of individuals inheriting a duplication from both parents. The two types were later obtained also from crosses analogous to those of the scheme outlined above.

Dp-bearing plants homozygous for *a-X1*, and those representing the *a-X1/a-X2* and *a-X1/a-X3* compounds, were produced by crossing the appropriate types on *a-X1/a*^{p} (cross-type (3) above). Thus in each of the three crosses, the *a-X/a-X* types desired were produced in a progeny which included sib plants of *a-X/a*^{p} for comparison. In each case, the *a-X/a-X* plants were distinctly smaller and in general later than their *a-X/a*^{p} sibs, and were characterized invariably by the occurrence of the characteristic chlorophyll-lacking sectors described above and pictured in fig. 1, B. The variegation type observed showed no consistent differences in the three *a-X/a-X* genotypes included.

These sectors, marked by absence of chlorophyll, in general gave no indication of loss of *A*, for the anthocyanin pigmentation within the sector was in almost all cases unaffected. Examination for anthocyanin effects cannot be made effectively in leaf blade tissue, since little anthocyanin is produced in this tissue. Many of the sectors identified in the blade extend into the leaf sheath, in which, with the proper complementary factors, anthocyanin pigmentation is strong. For pigmentation of the sheaths above the base, the complementary factor *B* is essential, and unless the complementary factor *Pl* is also present, pigmentation is absent in the portion of the sheath shielded from light by overlying tissue. Critical examination for anthocyanin effects in the sectors can therefore be made only in *B Pl* plants, and in some of the sectors of *B pl* plants. Most of the sectorial plants carried *B* and a substantial proportion had *Pl* also.

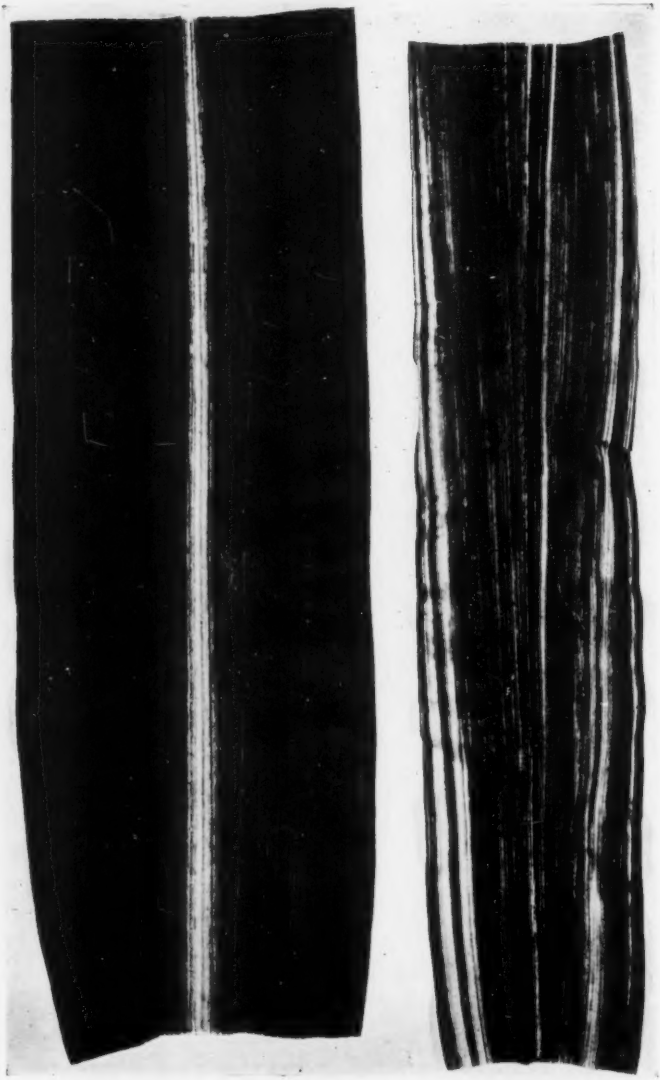
Among these, close examination was made for sectors lacking anthocyanin, with or without associated lack of chlorophyll. In plants heavily pigmented with anthocyanin in the sheath, small sectors lacking chlorophyll cannot always be detected in sheath tissue. The broader sectors found in the leaf blade, clearly marked by absence of chlorophyll, may usually be traced back



A

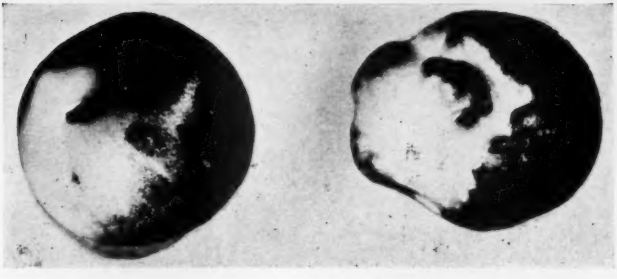
B

FIGURE 1. Characteristic variegated plant types. A. *a/a, Dp*. Note broad to narrow sectors lacking anthocyanin. There are no sectors lacking chlorophyll. B. *a-X1/a-X1, Dp*. Note narrowed sectors lacking chlorophyll. Very few of these sectors lack anthocyanin.



A B

FIGURE 2.—Leaves of plants shown in Fig. 1. A. *a/a, Dp.* No chlorophyll sectors. B. *a-X1/a-X1, Dp.* Numerous chlorophyll sectors.



A B

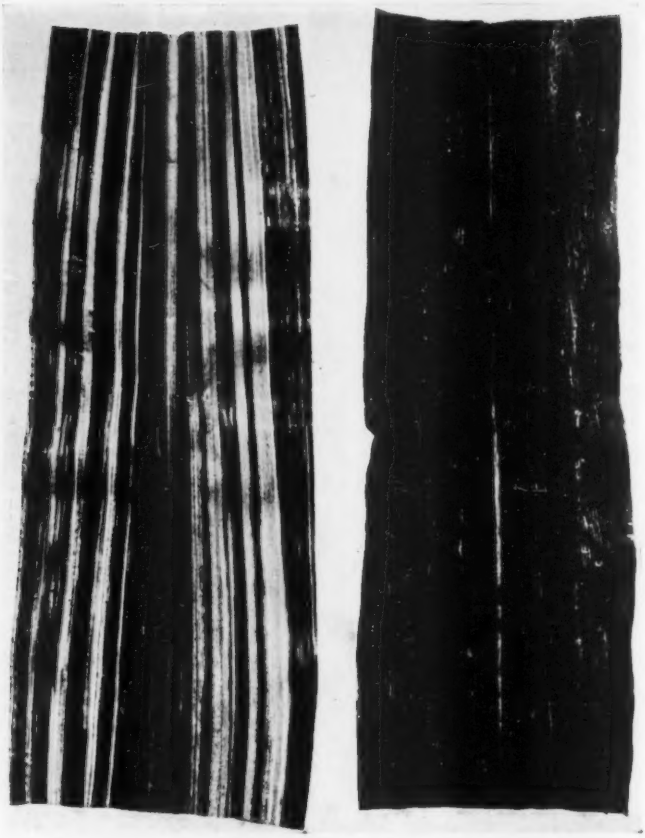
FIGURE 3. Characteristic endosperm mosaics. A. *a/a, Dp.* The mosaic sector is characterized only by the absence of anthocyanin pigmentation. B. *a-X1/a-X1, Dp.* The mosaic sector is characterized by degenerate endosperm tissue.

to the sheath, where even in the presence of strong anthocyanin the sector is recognizable by its lighter color resulting from the absence of chlorophyll. Such sectors are reasonably numerous, a single leaf sometimes providing a dozen or more. The counts were always made from only one leaf of any plant, to avoid including sectors of common origin in different counts.

It was found that in a large majority of the sectors lacking chlorophyll, anthocyanin pigmentation was present and apparently wholly normal, in comparison with adjoining tissue outside the sector. Much less frequently

A B

FIGURE 4. Sib plants in F_1 of *a-X3/a*^{p}×*a-X1/a-X3, Dp.* A. *a-X1/a-X3, Dp.* B. *a-X3/a-X3, Dp.*



A B

FIGURE 5. Leaf blades of plants shown in Fig. 4. A. *a-X1/a-X3, Dp*. B. *a-X3/a-X3, Dp*.

sectors lacking anthocyanin as well as chlorophyll were found. Usually such sectors were adjoined on one or on both sides by sectors lacking chlorophyll only, as if the sector characterized by loss of both anthocyanin and chlorophyll occurred within a larger sector initiated by loss of chlorophyll only. There were, however, other cases in which sectors showing loss of both anthocyanin and chlorophyll occurred without adjoining sectors showing loss of chlorophyll only.

In rare instances sectors lacking anthocyanin and not lacking chlorophyll occurred. The clearest case of this kind, observed in an *a-X1/a-X3, Dp* plant, was a sector about four mm in width, devoid of anthocyanin and clearly normal in chlorophyll. It was adjoined on one side by normal tissue with deep antho-

cyanin pigmentation, with a sharp line of demarcation between the pigmented and non-pigmented tissues. On the other side it was adjoined by a sector about six mm wide, in which chlorophyll was lacking. Within the latter sector there were several streaks of anthocyanin-bearing tissue, but otherwise anthocyanin was lacking in this sector also. Among sectors lacking anthocyanin, both in cases with and without chlorophyll, there is sometimes clear brown pigmentation similar to that found in a *a B Pl* plants.

These observations suggest that the sectors observed in the *a-X/a-X* plants result not from the loss of the duplication as a whole, but from losses of a portion only. If the *a-X* alterations represent deficiencies which are effectively cell-lethal, this is the result to be expected. Losses of the duplication as a whole, occurring in the course of development, would only eliminate the cell progeny of occasional cells of growing meristems. This might result in the reduced growth and distortion observed in the *a-X/a-X* plants as compared to their *a-X/a*^{p} sibs, but could not produce sectors of phenotypically distinguishable tissue. But if the duplication is capable of partial loss in mitosis, such as the diminution in ring chromosomes demonstrated by McCLINTOCK (1938), and if the cell-lethal effect of the *a-X* alterations is due to a locus different from the *A* locus or the chlorophyll-essential locus (here designated *W*), then partial losses could occur which would given viable sectors lacking chlorophyll or anthocyanin or both. The relative frequency of such sectors would depend upon the sequence of the genes concerned, relative to the centromere of the duplication fragment. The relatively low frequency of sectors lacking both chlorophyll and anthocyanin, and the fact that there are usually found adjoining sectors lacking chlorophyll only, suggests that a factor essential for cell-viability may be so located that losses of a segment including both *A* and *W* would ordinarily include the cell-viability factor, but a loss involving one of the two loci might be followed by a loss involving the other, without including the viability factor.

It should be possible, by detailed study of the cytological behavior of the fragment and of the relations of the various genetic effects in the sectors, to locate these factors more definitely, and to distinguish between the various viability effects involved. For our present purpose, the essential point is the demonstration that all three of the *a-X* alterations are characterized by the absence of distinct and separable genetic effects. This is evident from the varying types of sectors observed.

The phenotypic similarity of *a-X1/a-X1, Dp* plants to the compounds *a-X1/a-X2, Dp* and *a-X1/a-X3, Dp* is best tested in the progeny of crosses of the type *a-X1/a*^{p}×*a-X1/a-X2* (or *a-X3*), *Dp*. Here the seeds with degenerate sectors include two types, *a-X1/a-X1, Dp* and *a-X1/a-X2* (or *a-X3*), *Dp*. There was little basis in the appearance of the plants for any attempted classification of the two genotypes. In the case of *a-X1/a-X1, Dp* versus *a-X1/a-X3, Dp*, attempted classifications may be checked at flowering by pollen examination, for the plants of the latter class show about 40 percent subnormal pollen due to the effect of *a-X3* in the absence of *Dp*. These trials showed no relation of the attempted classifications to the presence or absence of *a-X3*. The three

Dp-bearing *a-X/a-X* genotypes including *a-X1* thus appear to be phenotypically indistinguishable.

Of the three remaining *Dp*-bearing genotypes, *a-X2/a-X2*, *a-X2/a-X3*, and *a-X3/a-X3*, the first two also are similar to the *a-X1* homozygote and compounds. These types, obtained in various progenies, were always recognizable as defective plants with chlorophyll-lacking sectors, similar to those previously described. The third, *a-X3/a-X3*, *Dp*, is distinctly different. This may be illustrated in the progeny of the cross *a-X3/a*^{p}×*a-X1* (or *a-X2*)/*a-X3*, *Dp*. Because of low transmission of *a-X3* by the female parent, this cross yields only a few seeds of the desired types per ear, but by pollinating several ears of *a-X3/a*^{p} by pollen from a single plant, small but adequate populations were secured. The results are as follows:

The seeds produced are mostly colored-pale mosaics. The remainder include some with clear degenerate sectors and some with no clear sectors, but usually with a slight pitting of the surface. In some cases no pitting is detectable, and the seed appears fully colored.

Plants grown from the seeds with degenerate sectors are typical *a-X/a-X* sectorial plants of the type previously described, and are indistinguishable from those previously found with *a-X1* or *a-X2*. These plants show the segregation of subnormal pollen associated with *a-X3*, but in proportions below 50 percent, as in the *a-X3* heterozygotes previously reported. They are thus identified as the *a-X1* (or *a-X2*)/*a-X3*, *Dp* class expected.

Plants grown from the pitted seeds or from the wholly colored seeds are of a new type, regularly defective in comparison with their *a-X/a*^{p}, *Dp* sibs and usually more defective than their *a-X1* (or *a-X2*)/*a X3*, *Dp* sibs. These plants usually show no sectors except for numerous minute streaks of chlorophyll-lacking tissue. Occasionally a small sector similar to those found in the other *a-X/a-X* types occurs, but ordinarily the plant is distinguishable only by its fine-streaked leaf blades and its generally defective development. A considerable proportion of these plants fail to survive to the flowering stage, but the majority survive to maturity and usually produce a few seeds. These plants show subnormal pollen in proportions approximating 80–85 percent, or subnormal and defective pollen approximating this total. They are thus identified as the *a-X3/a-X3*, *Dp* class expected. The contrasted types of *a-X/a-X*, *Dp* plants are illustrated in figs. 4 and 5.

Thus *a-X3* must differ from *a-X1* and *a-X2* in some viability factor or factors concerned in the development of the sectors produced as a result of loss or diminution of the duplication fragment. If it be assumed that *a-X3* involves deficiency of a viability factor or factors not involved in the other *a-X* alterations, it does not necessarily follow that sector survival would be appreciably affected, for the evidence indicates that the sectors observed with *a-X1* and *a-X2* result only from partial loss of the fragment. Such partial losses would not necessarily "uncover" the viability deficiency peculiar to *a-X3*. The observed types however indicate that this deficiency is so located that the more frequent types of partial loss which are capable of producing sectors in the usual *a-X/a-X* combinations do "uncover" it.

The interpretation applied may best be illustrated by considering the various classes of progeny in a cross of the type *a-X3/a*^{p}×*a-X1/a-X3, Dp*. The progeny includes four genotypes, which we may designate for convenience as classes I, II, III and IV as follows:

	GENOTYPE
I	*a-X1/a*^{p}, *Dp*
II	*a-X3/a*^{p}, *Dp*
III	*a-X1/a-X3, Dp*
IV	*a-X3/a-X3, Dp*

All classes of the progeny include the same *Dp* fragment (or, assuming that the fragment is constantly changing, equivalent samples from the mixture of *Dp* types present in the pollen grains of the plant used as male parent). The fragments present in the progeny plants therefore must undergo elimination and partial loss of the same kinds in plants of the four classes. The total effects of such losses are best indicated by classes I and II, for these carry a chromosome 3 free from viability defects (the *a*^{p}-bearing chromosome). Any loss of the duplication, in whole or in part, therefore will be without effect upon subsequent development of the affected tissue. In these classes, visible sectors result only from losses which include the *A* locus.

The frequent occurrence of large sectors lacking *A*^{b} in classes I and II shows that the *Dp*-fragment is undergoing frequent loss in development, including losses at early stages of development. These losses may be entire or partial, so long as they include the *A* locus. Presumably they include chiefly cases of loss of the entire fragment.

The nature of the sectors observed in class III plants shows that few, if any, such losses, when they occur in plants of this genotype, result in the production of visible sectors. Sectors lacking *A*^{b} are extremely rare. Since there is no reason to assume that these losses do not occur, we must postulate a genotype for the *a-X* alterations involved which prevents the development of these sectors. A factor (or factors) essential for viability, present in the normal chromosome 3 in the region covered by *Dp 3a*, must therefore be lacking in both *a-X1* and *a-X3*. Since *a-X2* compounds show the same behavior, it must be lacking also in *a-X2*. The location of this factor is such as to make possible its preservation when *W* and *A* are eliminated in successive losses, though it is commonly lost when *W* and *A* are lost simultaneously. The sectors involving loss of *W* and not of *A*, which make up the great majority of the sectors observed in plants of class III, result from a type of loss not detectable in classes I and II, since these classes are not genetically marked at the *W* locus.

An alternative considered in the interpretation of the variegation observed in the plants of class III was the possibility that the sectors observed are in fact deficient for *A*^{b}, in spite of the fact that they show anthocyanin. It might be supposed that the boundary of a sector deficient for *A* would not be sharply marked by absence of anthocyanin, since cells at the margin of the sector might include the pigment by reason of diffusion of anthocyanin or precursor substances from the adjoining non-deficient tissue. If the sectors were narrow

enough this might result in apparently uniform anthocyanin pigmentation throughout the sector. On this basis the sectors found in class III plants could be considered the result of the same losses as those found in classes I and II, and the loss responsible in both cases could be loss of the entire fragment. The reduction in size of sector might be considered to be the result of reduced growth in the *a-X/a-X* tissue, and the difference in phenotype the result of an effect upon chlorophyll development involved in the *a-X* alterations. It would still be necessary to postulate effects of the *a-X* alterations upon anthocyanin, chlorophyll, and growth, but no separation of these effects would be demonstrated by the variegation patterns observed.

This possibility is not supported by histological examination of the tissues within and adjoining the sectors. It requires the assumption that a very broad margin must be subject to pigmentation by diffusion, for some of the chlorophyll deficient sectors are eight to ten mm wide, and are uniformly colored by anthocyanin throughout. It is contradicted by the sharpness of the margins of the exceptional types of sector in which anthocyanin, or both anthocyanin and chlorophyll, are deficient, and especially by the fact that this margin is sharp on a side adjoining normal tissue as well as on a side adjoining a chlorophyll-deficient sector. We are forced to conclude that the sectors involving loss of chlorophyll alone represent a second type of loss, distinct from that involving loss of anthocyanin in classes I and II, and occurring frequently enough to produce a large number of sectors on each leaf.

The disappearance, or extreme reduction in size, of these sectors in plants of class IV, similarly shows an additional viability effect in plants of genotype *a-X3/a-X3*. Alterations in the *Dp* fragment of the "second type" mentioned in the preceding paragraph, when they occur in plants of *a-X3/a-X3*, *Dp* genotype, fail to result in sectors comparable to those found in other *a-X/a-X*, *Dp* plants. The chlorophyll-essential factor *W* must be deficient in *a-X3* as well as in *a-X1* and *a-X2*; otherwise the *a-X3* compounds *a-X1/a-X3* and *a-X2/a-X3* could not show the chlorophyll-deficient sectors. The absence of these sectors in the *a-X3/a-X3*, *Dp* plants must therefore be due to their failure to develop, through the action of some viability-affecting factor, or factors, distinguishing *a-X3* from *a-X1* and *a-X2*. The factor must be so located that its normal homolog tends to be lost from the duplication when *W* is lost.

The evidence secured through the use of the unstable duplication thus shows that the *a-X* alterations studied involve the loss of several genetically separable effects observable in somatic development, aside from any additional factors which may be involved in their various effects upon gametophytic survival and pollen development. All three of the alterations involve the loss of effects essential for anthocyanin production, chlorophyll production, and somatic viability. The results of these losses may be suppressed in different combinations by the different derivatives of the *Dp* fragment which are produced in the course of development of a *Dp*-bearing plant. All three effects are suppressed by the "intact" *Dp* fragment, which is maintained in *a-X/a-X*, *Dp* stocks and thus shielded against losses which would make it useless for cover-

ing the *a-X* alterations. The viability and anthocyanin effects are suppressed without suppression of the chlorophyll effect by one type of variant; the viability and chlorophyll effects are suppressed without suppression of the anthocyanin effect by another; and the viability effect is suppressed without suppression of the anthocyanin or chlorophyll effect by a third. Each of these effects may thus be separated from the other two. In addition, *a-X3*, by similar evidence, is shown to involve loss of an additional factor or factors essential for somatic development. This demonstration of the loss of several genetically separable factors in each of the induced alterations constitutes genetic proof of deficiency.

DISCUSSION

This study of the losses of *A* action induced by X-ray treatment gives no evidence of an effect of the treatment upon gene mutation. It shows that the segmental deficiencies induced in maize by X-ray treatment range to lengths so short as to be distinguishable from gene mutations only in exceptionally favorable material. Among a large sample of "*A* losses" the two selected for detailed study as most nearly approximating the expected effect of gene mutation proved to be deficiencies, distinguished only by the relatively minute length of the segment involved.

The background for the interpretation of these results may be briefly outlined as follows: X-ray treatment, as shown by earlier experiments concerned with the general mutation rate, results in a substantial increase in the frequency of point mutations. These are identified, in experiments with maize, as new Mendelizing characters observed in the F_2 of crosses in which one or both of the parents were X-rayed. The X-ray-induced point mutations found in maize prove to be wholly recessive in all cases. They include cases in which the mutant segregates in the expected 3:1 ratio, and also cases in which the ratio is reduced to varying degrees, usually by reduced transmission through male germ cells. The same X-ray treatments produce in large numbers chromosomal derangements of various sorts. There is no appreciable tendency of the induced mutations to occur at points of chromosome interchange, a feature in contrast with the results of comparable experiments with Drosophila. The primary question is whether the point mutations observed represent in the main the result of gene mutations or the result of extra-genic alterations. If the results indicate the latter to be true, a second question arises: Do the X-ray-induced mutations include any cases in which gene mutation must be assumed to have occurred?

The presumption that the point mutations in general represent gene mutations is based upon the assumption that deficiencies would ordinarily be lethal to the gametophyte, or at any rate would be so weakened in haplo phase as to fail in transmission through pollen. If this were so their recessive effects could not appear in F_2. This assumption becomes more and more questionable as cases are found of demonstrable deficiencies transmitted through the gametophyte generation. Deficiencies now known range in gametophytic effect from total abortion of both male and female gametophyte through successive levels

of development ranging to apparently normal development and normal functioning. Moreover the cases demonstrated as deficiencies range to the limit of cytological or genetic identification. The assumption that deficiencies do not occur below these limits of detectability is wholly unwarranted.

In selecting individual cases for detailed study as possible gene-mutations, it was assumed that the action of *A* is not essential for normal gametophyte development, and therefore that any deleterious effects of an observed *A* loss upon gametophyte development indicate that other loci are also involved. This assumption was based upon the normal gametophyte development found in all known *A* alleles, including several spontaneous mutations observed from the first generation following their occurrence. The assumption is confirmed by the results of this study, showing two deficiencies of *A* which permit normal development of both the male and the female gametophyte.

Among the population of *A* losses observed in F_1, then, we may expect that the major deficiencies including the *A* locus will have visible effects upon gametophyte development, though there may also be minor deficiencies with no detectable gametophytic effect. The *A* losses due to alterations with visible gametophytic effects will include most of the deficiencies, and may include losses of *A* effect due to other extra-genic alterations, but will not include any of the *A* losses due wholly to gene mutation. The *A* losses without visible gametophytic effects will include all of the gene mutations (except such as might occur from a single alteration involving both gene mutation and chromosomal derangement), and may include also some of the deficiencies and other extra-genic alterations.

Unfortunately, it is not possible in general to determine from inspection of the mutant individuals observed in F_1 whether or not the alteration concerned has gametophytic effects. The *A* loss observed is induced by X-ray treatment, and the coincidental occurrence of an independent chromosomal derangement induced by the same treatment may produce in the same F_1 plant a gametophytic effect not due to the alteration involving *A*. It is technically impossible to extract the *A* alteration in each case for individual study, not only because of the labor involved, but because in many cases the single plant concerned is sterile. The possibility remains therefore that among the population of *A* losses observed there were instances of gene mutation of *A*, which by reason of coincident but independent chromosomal alterations were accompanied by gametophytic or developmental defects.

The nature of the deficiencies identified in *a-X1*, *a-X2*, and *a-X3* is of interest in relation to the question of distinguishing between deficiencies and gene mutations among the point mutations at miscellaneous loci, which are induced in maize by X-ray treatment. It is clear that deficiencies including three or more gene loci may be too minute for cytological detection. In spite of the extremely sensitive reaction of crossover frequency to minute deficiency in maize, the results with *a-X1* show that such deficiencies may occur without pronounced effect upon crossover frequency. Genetic detection of the deficiencies in this case was facilitated by the fact that loci affecting chlorophyll development and somatic viability occur very close to the *A* locus, but there

is of course no reason to assume that such convenient linkages would occur with another gene similarly studied. There may be many genes with phenotypic effects, which are so located that deficiencies of comparable length could remove them without removing other detectable genes. Such deficiencies would be indistinguishable from gene mutations.

The somatic viability locus deficient in all three *a-X* alterations studied is apparently close enough to *A* to ensure that point mutations of *A* would be extremely infrequent in experiments of the usual type on X-ray-induced mutation of miscellaneous genes. So far as we know, no X-ray-induced mutations of *A* have previously been found. None of the three *a-X* alterations here described would appear in F_2 in such an experiment, since all are zygotically lethal, due to the associated viability factor. Although the loci yielding mutations under X-ray treatment appear to be a random sample, there are of course many known genes for which X-ray induced mutants have not been found. It is not improbable that with significant comparisons different loci would show large differences in susceptibility to X-ray-induced mutation, due to this cause.

If no viability factor were included in the *a-X* deficiency-segments, the alterations would segregate in F_2 as mutant alleles of *A* with effects upon both anthocyanin development and chlorophyll development. Such alterations with diverse effects are commonly regarded as gene mutations, particularly if they occur at loci not already identified by a consistent group of alleles. The X-ray induced mutant *et*, described in connection with the crossover experiments in this paper, is perhaps an analogous case. It affects two characters not obviously related, scarring of the endosperm surface and chlorophyll development in the seedling stage. The homozygote is fully viable and fertile, pollen development is normal, and the mutant is transmitted through both male and female gametophytes.

The three deficiencies designated *a-X1*, *a-X2*, and *a-X3* apparently represent segmental losses of different extent in each case. *Df a-X3* must be assumed to include some locus or loci not involved in the others, to account for its visible effect on pollen development and its much more extreme reduction in gametophyte survival. The additional somatic viability factor or factors involved in *a-X3*, as indicated by the studies with the unstable duplication, may or may not represent the same loci. Similarly *a-X2* must differ from *a-X1*, to account for its much more pronounced effect upon crossing over, as well as its consistently lower gametophytic transmission.

Since these three deficiencies represent breaks at different points in the normal gene sequence, it is reasonable to infer that a larger sample would include deficiencies of smaller extent. A deficiency including the *A* locus and not including the viability and chlorophyll-essential loci here involved would be viable in homozygotes and would be identical in phenotypic effect with the standard recessive allele *a*.

SUMMARY

1. X-ray-induced losses of the phenotypic effect of *A* (here designated "A losses") were identified in F_1 populations produced by the use of X-rayed

pollen. Among about 415 *A* losses observed, only two were normal plants free from segregating pollen defects.

2. The induced germinal alterations responsible were extracted for study in these two cases (*a-X1* and *a-X2*). The alteration involved in an *A* loss showing segregation of subnormal but not aborted pollen was also extracted for comparison (*a-X3*).

3. All three alterations are haplo-viable but with reduced transmission thtough the gametophyte generation. *a-X1* is usually transmitted with normal frequency through female gametophytes, and with about 50 percent of normal frequency through male gametophytes. *a-X2* usually shows about 65 percent of normal female transmission and about 25 percent of normal male transmission. *a-X3* usually gives about 20 percent of normal female transmission, and is not at all transmitted through pollen.

4. Homozygotes of *a-X1* and *a-X2*, and the compounds of *a-X1*, *a-X2*, and *a-X3* in all three possible combinations, are zygotically lethal.[1]

5. Plants heterozygous for *a-X2* and *a-X3* show greatly reduced crossing over in the adjacent region of the chromosome, while plants heterozygous for *a-X1* show approximately normal crossing over in the same region.

6. Cytological examination at pachytene in plants heterozygous for *a-X2* and *a-X3* shows no visible deficiency, intercalation, or inversion, and chromosome pairing in the region involved is not visibly abnormal.

7. By the use of an unstable duplication, *Dp 3a*, covering the region concerned, the various *a-X*/*a-X* homozygotes and compounds were produced, and the sectors resulting from elimination or partial loss of the *Dp* fragment were studied for evidence concerning the factors involved in the various *a-X* alterations. This study showed that all three alterations are deficient for factors affecting anthocyanin production, chlorophyll production, and somatic viability, and that *a-X3* is deficient for an additional factor or factors affecting somatic viability.

LITERATURE CITED

EMERSON, R. A., and E. G. ANDERSON, 1932 The *A* series of allelomorphs in relation to pigmentation in maize. Genetics **17**: 503–509.

FOGEL, SEYMOUR, 1946a Gene action and histological specificity of pigmentation patterns of certain *R* alleles. Genetics **31**: 215–216.

1946b Gene action and the course of anthocyanin synthesis in certain *R* alleles. Genetics **31**: 215–216.

LAUGHNAN, JOHN R., 1946 Chemical studies concerned with the action of the gene A_1 in maize. Genetics **31**: 222.

MCCLINTOCK, BARBARA, 1931 Cytological observations of deficiencies involving known genes, translocations and an inversion in *Zea mays*. Missouri Agric. Exp. Sta. Res. Bul. 163, pp. 1–30.

1933 The association of non-homologous parts of chromosomes in the midprophase of meiosis in *Zea mays*. Z. Zellf. mik. Anat. **19**: 191–237.

1938 The production of homozygous deficient tissues with mutant characteristics by means of the aberrant mitotic behavior of ring-shaped chromosomes. Genetics **23**: 315–376.

1944 The relation of homozygous deficiencies to mutations and allelic series in maize. Genetics **29**: 478–502.

[1] See footnote, p. 280.

RHOADES, M. M., 1941 The genetic control of mutability in maize. Cold Spring Harbor Symp. Quant. Biol. **9**: 138–144.

STADLER, L. J., 1933 On the genetic nature of induced mutations in plants. II. A haplo-viable deficiency in maize. Missouri Agric. Exp. Sta. Res. Bul. 204. pp. 1–29.

1935 Genetic behavior of a haplo-viable internal deficiency in maize. (Abst.) Am. Nat. **69**: 56–57.

1940 Etched endosperm-virescent seedling. Maize Genetics Cooperation News Letter **1940**: 26.

1941 The comparison of ultraviolet and X-ray effects on mutation. Cold Spring Harbor Symp. Quant. Biol. **9**: 168–177.

1942 Some observations on gene variability and spontanous mutation. The Spragg Memorial Lectures on Plant Breeding (third series). Mich. State Coll., East Lansing.

1944 The effect of X-rays upon dominant mutation in maize. Proc. Nat. Acad. Sci. **30**: 123–128.

1946. Spontaneous mutation at the *R* locus in maize. I. The aleurone-color and plant-color effects. Genetics **31**: 377–394.

STADLER, L. J., and SEYMOUR FOGEL, 1943 Gene variability in maize. I. Some alleles of *R* (*R*^{r} series). Genetics **28**: 90–91.

1945 Gene variability in maize. II. The action of certain *R* alleles. Genetics **30**: 23–24.

STADLER, L. J., and HERSCHEL ROMAN, 1943 The genetic nature of X-ray and ultraviolet induced mutations affecting the gene *A* in maize. Genetics **28**: 91.

OBSERVATIONS ON CROSSING OVER INDUCED BY X-RAYS IN THE MALES OF DROSOPHILA

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THE induction of crossing over in the male of *Drosophila melanogaster* has been reported by PATTERSON and SUCHE (1934), who concluded that it occured in the growth stages of the spermatocytes. This conclusion was based on the assumption that, were the crossing over taking place in pre-spermatocyte stages (that is, spermatogonia or stem cells), its rate should be highest in the youngest age irradiated, since a larger proportion of the gametes to be sampled would be represented by such stages. They found the rate in 40–52 hour larvae to be some six times greater than the rate in 24–36 hour larvae.

FRIESEN (1934) has likewise reported the production of crossing over in the male by irradiation. In a later paper (1936), on the basis of the occurrence of clusters of similar crossovers in the progeny of individual males, he concluded that crossing over must be occurring in a stage earlier than the spermatocyte; he assumed it to be spermatogonial crossing over. If FRIESEN is correct in assuming that crossing over in one cell results in all of the crossovers recovered in one cluster, then in some cases the large size of the clusters makes it likely that crossing over is being induced in one of the apical stem cells. An attempt will be made in this paper to explain the occurrence of clusters without assuming spermatogonial (or stem cell) crossing over.

Both PATTERSON and SUCHE, and FRIESEN (1937) describe the crossover chromosomes as being normal, with few exceptions. The few lethals found were not associated with the point of break. FRIESEN'S studies included examination of the crossover chromosomes in the salivary glands. On the basis of the absence of lethal position effects, PATTERSON and SUCHE concluded that induced crossing over could not be the effect of chromosome breakage by X-rays, but rather is due to a possible alteration of the rate of maturation. However, if the crossover chromatids are broken at identical loci, the absence of lethal position effects might be expected. Thus if the breakage is produced by irradiation in the same manner as in aberrations, the absence of lethal position effects would show such effects to be the result of rearrangement and not of breakage.

If induced crossing over is due to orthodox chromosome breakage, and if the majority of the crossover chromosomes recovered are essentially normal as reported, then the possibility suggests itself that the breakage of the two chromatids involved is accomplished by a single ionizing particle. The primary purpose of this paper is to open up the question of the relationship between dosage and frequency of induced crossing over to see if this relationship indicates breakage by a single particle.

EXPERIMENTAL PROCEDURE

Larvae heterozygous for the third chromosome multiple recessive stock "*3ple*" (*ru h st* p^p *ss* e^s) were irradiated when 40–52 hours old, the age range where PATTERSON and SUCHE found the highest incidence of crossing over. On emerging, each of the males was mated individually to four homozygous "*3ple*" virgin females. The F_2 offspring emerging during the first three days were counted.

To reduce the errors of dosage measurement, the sample to receive the half dose in each case was divided into two halves, one being substituted for the other mid-way through the treatment. The peak voltage employed was 56 kv; the radiation was filtered through 1 mm of aluminum. The dose rate was approximately 100 r/minute, as determined by the use of a Victoreen r-meter.

EXPERIMENTAL RESULTS

The results are summarized in the accompanying tables, with table 1 showing the frequencies of all crossovers at each dose employed. While the lower

TABLE 1

Frequency of crossing over.

SERIES	DOSE	NUMBER MALES TESTED	NUMBER PRODUCING CROSSOVERS	NON-CROSSOVERS	TOTAL SINGLES	TOTAL DOUBLES	TOTAL COUNTED	PERCENT CROSSING OVER	LIMITS OF ERROR*	
1	500 r	354	58	18,744	80	3	18,827	.46	.37	.56
	1000 r	180	50	9,030	59	4	9,093	.74	.54	.88
2	1000 r	198	47	11,451	70	1	11,527	.63	.48	.86
	2000 r	22	6	891	11	—	902	1.22	.62	2.20
	Control	128	1†	9,937	1†	—	9,938			

* Calculated by the method of STEVENS (1942) with a significance of .05.
† One *ru* male which when crossed with homozygous "*3ple*" females produced only wild and "*3ple*" offspring.

doses had little apparent effect on the viability and fertility of the imagos from the irradiated larvae, adults failed to emerge in a very large proportion of those receiving 2000 r. Many of the latter which did emerge died shortly thereafter, or at best failed to produce offspring. Approximately five percent of the survivors produced offspring, producing almost as many per male as did those receiving the lower doses. In the controls and in the series receiving 500 r and 1000 r, approximately 90 percent of the males tested were fertile. The calculations of limits of error in table 1 follow the method of STEVENS (1942).

Table 2 shows the distribution of crossovers in the samples. Over one half of the crossovers recovered were in clusters of from two to five crossovers each. In practically all of these cases the crossovers in each cluster were similar—

TABLE 2

Distribution of crossovers. Vertical columns show total number of males producing 0, 1, 2, 3, 4, 5 crossovers.

DOSE	NUMBER OF CROSSOVERS FROM A SINGLE MALE					
	0	1	2	3	4	5
500 r	296	40	13	4	—	1
1000 r	281	70	19	6	1	1
2000 r	16	2	3	1	—	—
Totals	593	112	35	11	1	2

TABLE 3

Composition of clusters with dissimilar crossovers.

NO. OF CLUSTERS	CROSSOVERS
1	*p^p, p^p ss e^s*
1	*ru h, ru h st*
1	*ru h, p^p ss e^s*
1	*p^p ss e^s, ru h st p^p e^s*
1	*ru, ru h st*
2	*ru, p^p ss e^s*
1	*p^p ss e^s, e^s*
1	*st, ru h st, p^p ss e^s*
1	*st p^p ss e^s, ru h st, p^p ss e^s*
1	*ru h st, p^p ss e^s, e^s*
1	*ru h st, p^p ss e^s, 2 ru h st p^p*
1	*3 ru h, 2 p^p ss e^s*

produced by crossing over in the same region with both classes being freely represented. The exceptional cases are listed in detail in table 3.

Of the 35 clusters of two crossovers each, 27 were similar pairs. One cluster consisted of a double and a related single. Two clusters consisted of single crossovers in which the regions of exchange were adjacent. One was made up of a double involving two adjoining regions and a single involving a third adjoining region. Four of the pairs were single crossovers involving non-adjacent regions.

Of the 11 clusters of three crossovers each, eight consisted of three similar

crossovers. One consisted of a double with two related singles. One consisted of two similar singles, with the third single involving an adjacent region. One was made up of a pair of similar singles, with a third single involving a non-adjacent region.

The one cluster of four singles involved two adjacent regions of crossing over, each being represented twice. One of the clusters of five was made up of five similar singles, while the other involved two adjacent regions, one being represented twice the other thrice.

Table 4 shows the types of doubles recovered, and compares them with the doubles reported by PATTERSON and SUCHE. Eight double crossovers were recovered in this experiment, in all cases involving two adjacent regions of exchange. The 12 doubles reported by PATTERSON and SUCHE are quite similar in this respect, with the exception of four cases, three of which involved the distal

TABLE 4

Types of double crossovers recovered.

PRESENT DATA:		DATA OF PATTERSON AND SUCHE:	
REGIONS	NUMBER	REGIONS	NUMBER
		ru-h, h-th	1
		h-th, th-st	1
h-st, st-p^p	2	*th-st, st-cu*	2
st-p^p, p^p-ss	4	*st-cu, cu-sr*	3
p^p-ss, ss-e^s	2	*cu-sr, sr-e^s*	1
		ru-h, e^s-ca	3
		h-th, st-cu	1
Total	8	Total	12

regions of the two arms of the third chromosome. One of their doubles involved two regions of exchange which were neither adjacent nor in opposite arms of the chromosome approximately equidistant from the centromere.

Thus, both the doubles and the clusters of dissimilar singles indicate considerable restrictions on crossing over, making adjoining regions the most likely possibility if two regions of crossing over are to be represented in the progeny of an individual male. The relative frequencies with which any two exchanges involving different regions are recovered are not dependent simply on the frequencies of crossing over, as in normal crossing over, since on this basis many of the possibilities which are not realized would be equally likely to occur as those which actually do.

The restrictions on the occurrence of crossing over are further indicated by the failure of doubles to increase at higher doses much more rapidly than do the singles. If crossing over can occur at the time of irradiation of a given cell in all parts of the chromosome, with increasing dosage (therefore with increasing frequency of crossing over) the probability of two simultaneous exchanges would be increased, and the ratio of singles to doubles should be decreased.

Table 1 shows 28 singles/double at 500 r and 25.8 singles/double at 1000 r—essentially the same.

Table 5 shows the distribution of crossing over by regions. The results for each dose show a similar distribution of crossing over, with approximately 60 percent of the observed crossing over taking place in the *st-p*^{p} region.

DISCUSSION

While the question of time of crossing over in the male is of no great significance to the main question of relationship of frequency to dosage, the facts at present available concerning X-ray induced crossing over can be explained

TABLE 5

Percentage of total crossing over for each marked region.

DOSE	REGIONS				
	ru-h	*h-st*	*st-p*^{p}	*p*^{p}*-ss*	*ss-e*^{s}
500 r	3.5	15.1	60.5	12.8	8.1
1000 r	9.3	10.8	63.3	9.3	7.2
2000 r	27.3	—	72.7	—	—

without assuming spermatogonial crossing over. The objections given to spermatogonial crossing over do not rule it out entirely; at the same time they certainly add no support to the idea. I propose the following explanation of crossing over occurring in the growth stage of the spermatocyte, based on the parallels between cytological observations and the characteristics of clusters described above.

LEAGUE (1931) states that some larvae will show growth stages, while others of similar age will show none, the space being occupied by second spermatocytes and spermatids. This corresponds to the production of several crossovers by some males, none by others.

The crossovers making up one cluster are most often alike, but when they differ they frequently involve adjacent regions of crossing over, indicating the restriction of crossing over at any time to a very short region. If crossing over is taking place in the spermatocyte, then crossing over of essentially the same type must be taking place simultaneously in several cells to produce these clusters. Both HUETTNER (1930) and LEAGUE agree in their descriptions of spermatogenesis that the Drosophila testis shows cyst formation, although the cysts are described as being weak, and walls are lacking. All of the cells in one cyst develop more or less synchronously, which would indicate that they would be subject to similar restrictions. If crossing over were then induced in several cells, several similar crossovers might be recovered.

Thus we can think of a short region in the chromosome which is competent to have crossing over induced in it, with synapsis presumably being the primary condition necessary for such competence. Possibly such competence is

gained first in the vicinity of the centromere, extending distally as it is lost proximally. This would account for the three doubles of PATTERSON and SUCHE involving the most distal regions. On this interpretation, the frequency of induced crossing over in a region is a function of the duration of competence in the region rather than of the absolute length of the region. The similarity of induced crossing over in male and female suggests that somewhat similar conditions might exist in the female.

Since only a short region of the chromosome is competent at any time, the production of two exchanges during any short interval usually would be undetectable, since they would seldom involve two separate marked regions. Thus a doubling of the actual rate of exchange would not result in a doubling of the observed rate of crossing over, due to the increased probability of getting two exchanges in a single marked region. If the two breaks resulting in crossing over are due to the passage of a single ionizing particle, then the frequency of exchanges should show a straight line relationship to dosage. However, the observed rate of crossing over should show such a straight line relationship only at very low doses, levelling off as an upper limit is approached. The upper limit would depend upon the percentage of sperm in the sample which arose from cells which were competent at the time of irradiation.

On the basis of the observed rates at doses of 500 r and 1000 r, we may calculate such an upper limit to be approximately 1.2 per cent. The expected frequency of crossing over at 2000 r would be about 1.0 percent; the value actually obtained, 1.22 percent, lies fairly close but is obviously none too reliable due to the small numbers involved. It would be of some value to see how well the frequencies at lower doses fit such a theoretical curve.

None of the results here discussed are inconsistent with the interpretation that crossing over induced by X-rays is the result of direct breakage of the chromosomes. The frequency/dose relationship is that which would be expected on the basis of breakage of two chromatids by a single particle. This is in agreement with observations on isochromatid breakage in Tradescantia (LEA 1947).

The interpretation of crossing over as a result of chromosome breakage is further reenforced by the recent report of LEFEVRE (1947) which indicates that, since fast neutrons are "relatively much more effective per ionization than gamma rays" in inducing somatic crossing over, such induced somatic crossing over must be a "direct" effect of the radiations, resulting in chromosome breakage. LEFEVRE concludes that the two breaks are produced independently, since the frequency is dependent on the dose rate. If this is the case, crossover chromosomes would be produced with small duplications and deficiencies, which might account for the statement, "Evidence was found that mosaic size and age at exposure are not simply related. Twin mosaics in eosin-white females showed great inequality in size." If this interpretation should prove to be appropriate, somatic and germinal induced crossing over are not identical. It is not appropriate at this time to make any extensive comparisons between the two.

SUMMARY

Induced crossing over in the male is quite restricted in that only a short region of the chromosome can be involved at any time. This is indicated by the preponderance of doubles involving adjacent regions of crossing over, as well as by the occurrence of clusters of crossovers involving adjacent regions.

The frequency of double crossing over failed to increase greatly with increased dose. This is to be expected if crossing over is restricted to a region shorter than one marked region.

The occurrence of several crossovers in the progeny of some males and absence from the progeny of others is correlated with the presence of growth stages in some testes and absence from the testes of other males of similar age.

The cluster effect may be correlated with the similarity of stage of maturation of all cells in a cyst. All cells in one cyst might be similar in their crossing over restrictions at any time.

It is therefore suggested that the assumption that crossing over is being induced in the spermatogonia is unnecessary.

Induced crossing over may be interpreted as a result of chromosome breakage by X-rays, with the frequency/dose relationship indicating that both breaks involved in one exchange are produced by a single particle.

The absence of lethal position effects does not rule out the explanation of direct breakage by X-rays if both chromatids are broken at the same locus.

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LITERATURE CITED

FRIESEN, H., 1934 Künstliche Auslösung von Crossing-over bei Drosophila-Männchen. Biol. Zbl. **54**: 65–75.

1936 Spermatogoniales Crossing-over bei Drosophila. Z.I.A.V. **71**: 501–526.

1937 Mechanism of crossing-over in males of *Drosophila melanogaster*. J. Genet. **35**: 141–150.

HUETTNER, A. F., 1930 The spermatogenesis of *Drosophila melanogaster*. Zeit. f. Zellf. u. mikr. Anat. **11**: 615–637.

LEA, D. E., 1947 Actions of radiations on living cells. 402+xii pp. Cambridge University Press.

LEAGUE, B. B., 1931 The development of the male gonad and the meiotic phenomena in *Drosophila melanogaster*. (Doctoral thesis (MS), The University of Texas.)

LEFEVRE, GEORGE, JR., 1947 The relative effectiveness of fast neutrons and gamma rays in producing somatic crossing over in Drosophila. (Abstract.) Rec. Genetics Soc. America **16**: 40.

PATTERSON, J. T., and SUCHE, M. L., 1934 Crossing over induced by X-rays in Drosophila males. Genetics **19**: 223–236.

STEVENS, W. L., 1942 Accuracy of mutation rates. J. Genet. **43**: 300–307.

THE NATURE OF COAT COLOR DIFFERENCES IN MINK AND FOXES*[1]

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INTRODUCTION

COAT colors in laboratory mammals such as the mouse, rat, guinea pig and rabbit have long been used in genetic studies involving the physiology and transmission of genes. Horses, cattle, swine, sheep, goats and other domestic animals also present an array of different coat colors, but with the exception of the use of a particular color or color pattern as a breed marker, differences in pigmentation probably have little or no economic value in these forms. The situation is just the reverse with regard to the fur bearing animals raised in captivity; the value of the pelt is considerably influenced by color phase. Although color has always been one of the most important factors in determining pelt value, the fur trade became color phase conscious to a marked degree with the appearance of the first platinum silver fox pelts on the New York market in 1940. Since that time, mutant colors in mink and foxes have been in great demand.

The studies reported here deal with some quantitative and qualitative differences in pigmentation of the pelage of the silver, platinum and pearl color phases of the red fox (*Vulpes vulpes* L. = *V. fulva* species), and the dark, platinum and pastel color phases of the mink (*Mustela vison* Peale and Beauvois). The mutant color phases mentioned result from single gene substitutions, and so far as is known, all of the individuals contributing fur samples differed from their respective standard ranch-bred color phases only at the mutant locus indicated.

The main objective of the study was to isolate some of the links in the chain of events which intervene between the gene and its end point. This information should have value in studies of gene physiology. Three methods of approach to this problem have been considered: (1) histological aspects of guard hair pigmentation, (2) quantity of melanin pigment production as determined by weight, and (3) measurement of pigment granule length.

Coat color in mammals is generally attributed to melanins, organic compounds containing nitrogen, usually dark in color, and characterized by chemical inactivity. Observations such as the persistence of color in mutant spots or areas, and in transplantation experiments indicating the local pro-

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duction of pigment, and the resulting belief that the chain of reactions between gene and character is relatively short for mammalian coat color genes have led to many genetic analyses of the pigments produced in different genotypes of several mammals. WRIGHT (1942) has used the coat colors of the guinea pig as an example of some of the kinds of relations found between genes and characters.

The literature dealing with one or another phase of pigmentation is extensive and a number of investigators have given considerable attention to reviews of the literature. Since E. S. RUSSELL (1946) has given a rather complete selective review of the literature concerned with mammalian pigmentation, no attempt will be made at duplication here.

DESCRIPTION OF COLOR PHASES

Foxes

Most of the foxes raised in capitivity for their pelts are color phases of the American wild red fox (*Vulpes vulpes*, L.). A detailed description of the wild red fox and the black mutant that is the standard fox of the ranching industry has been dealt with elsewhere by COLE and SHACKELFORD (1943) and will not be repeated here except to state that in the black fox the red of the guard hairs and the albescence of the "white" areas of the wild red fox have been replaced by black. Two phenotypically similar mutations to black occurred in the wild red foxes of North America prior to the rise of the ranch breeding industry (ASHBROOK 1937); both are almost completely recessive to their respective non-mutant alleles, the one occurring in the Eastern United States and Canada designated as the Eastern Standard black, and the one occurring in Western Canada and Alaska as the Alaskan black.

The use of the term "black" was soon abandoned by the fur trade in favor of "silver" because of the exclusiveness, among fur bearers, of the type of silvering characteristic of the red fox (figs. 1a and 7 Dc). In most mammals, silvering refers to a sprinkling of white guard hairs throughout an otherwise colored pelage. The silvering of the red fox is dependent upon the portion of guard hairs with pigment-free subterminal bars scattered over the body (fig. 7Dc). Silvering appears to be inherited independently of color phase. KELLOGG (1941) interpreted degree of silvering to be dependent on several factors. Hereafter, the term "silver" will be used to designate the black color phase of the red fox.

Silver foxes long had such a hold on the trade that when a mutant color did appear, it was usually disposed of summarily as indicating lack of purity in the standard stock. In 1938 TUFF described a new type being raised in Norway which he referred to as "the platinum character in the silver fox." The general color of the platinum is lighter than that of the silver due not only to the grayish tone of the colored area but also to the smaller pigmented area. One of the most characteristic things about platinum is the considerable amount of white spotting that always accompanies it. MOHR and TUFF (1939) have well described it as follows: "White snout, a blaze along the nose and forehead

joining with a white color around the neck. The breast and a broad stripe on the belly are white and the same is true of the legs and the distal part of the tail. The size of the markings is somewhat variable, particularly on the legs, and the white collar may be lacking in dark individuals." It might be added that the amount of white on the face and the other parts of the body may in some cases be so small that the classification by phenotype might be doubtful as an index of genotype if it were not for the other characteristics of the platinum.

MOHR and TUFF (1939) presented breeding records clearly indicating "that the platinum character is due to an ordinary dominant gene," and suggested that it might be lethal in the homozygous condition. COLE and SHACKELFORD (1943) have discussed the occurrence and breeding histories of three separate mutations to platinum in the United States and Canada, which are presumably the same as the Norwegian platinum. Their data fully confirm the conclusions of MOHR and TUFF. White pups which are dead at birth or die shortly after, are occasionally found and are taken to represent individuals of the homozygous dominant class which have survived longer than is usual or by chance have been found. JOHANSSON (1947) has analyzed data from Swedish fox farms, and also concludes that the platinum character is due to an autosomal dominant gene which is lethal in the homozygous condition. Based on the results of limited experimental matings of platinum×platinum, however, he does not believe that homozygosity for the dominant mutant gene necessarily produces a white pelage.

A mutant coat color in the fox which is presumably homologous with maltese in the cat and the dilute character in other mammals is called pearl platinum, or simply pearl, by fox breeders. Pearl differs from the standard silver only in the general dilution of the black which gives the characteristic "blue" of this color phase; the pelage is a bluish gray throughout, and the eyes, nose, lips and claws are distinctly lighter than in the silver fox. BOWNESS (1944), who appears to be the first to have collected the breeding histories from several ranches on which pearl foxes were originally bred, says that LA DUE recalls having seen pearls in Minnesota as early as 1924. Previous to the popularization of the Norwegian platinum and the resulting general search for mutant color phases among silver foxes, however, pearls had occurred on several ranches in the United States and Canada and the character was understood among ranchers to be the result of homozygosity for a simple recessive gene. DEAKIN (1942) reported two different mutations in Canada which produce the pearl coat color. The coat colors resulting from these different mutations are so similar in appearance that they are phenotypically indistinguishable.

Mink

Domestic mink have been derived from the geographical races of the American mink (*Mustela vison*, Peale and Beauvois). The standard color of ranch-bred mink is similar to that of the wild mink; the pelage is usually dark brown, but may vary from light brown to near black. A stripe down the back, the tail and feet are darker than other parts of the body. The eyes, nose and claws are dark brown, and there are often white spots on the chin, throat, breast and

belly, although some individuals are free of all white markings. A number of color variations have arisen from ranch-bred mink; SHACKELFORD (1941), SMITH *et al.* (1941) and CASTLE and MOORE (1946) have described most of them and discussed the type of inheritance involved in each case.

Platinum[2] was the first mutant color phase to command the attention of mink breeders. This color phase had been observed in the wild, however, and the first platinum garment is reported to have been made from the pelts of animals taken by trappers over a period of several years. Platinum appears to have occurred first in captivity on the ranch of Mr. WILLIAM WHITTINGHAM of Arpin, Wisconsin, in 1929, but shortly thereafter made its appearance on several other ranches in the United States and in Canada. The pelage of the platinum mink is a light bluish gray in color. The claws, nose and lips are also lighter than in the dark mink. In other respects it is similar to the dark mink; the eyes are dark brown, ventral white spots are usually evident, and a stripe down the back, the tail and feet are darker than other parts of the body. The platinum character is due to homozygosity for an autosomal recessive, and is presumably homologous with pearl in the fox and the dilute character in other mammals.

Pastel, blond, and chocolate are a few of the names that have been applied to a light brown color phase in mink. This is similar in appearance to the "chocolate" mutation in other mammals.[3] The eyes, nose and claws are distinctly lighter than in the dark ranch-bred or average wild mink. It is often very difficult to distinguish pastel pelts from those of the lighter mink caught in the wild, which indicates that this color phase may have appeared in the wild and not been noticed. Since ranch-bred mink have been selected toward the dark brown to near black end of their range in coloring, no difficulty is experienced in distinguishing the pastel from dark mink under ranch conditions. The pastel character results from homozygosity for a simple recessive gene, and like most of the recessive color phases in both foxes and mink has occurred on several ranches in the United States and Canada. SHACKELFORD and COLE (1947) have reported an abnormal behavior pattern termed "screw neck" which is sometimes associated with this color phase.

HISTOLOGICAL ASPECTS OF PIGMENTATION

Several investigators have used the histological approach as an aid in determining the physical basis for color differences in hair. DURHAM (1904) noticed that in dilute mice the color of the pigment granule was unchanged from that of black animals, but that the pigment appeared to be clumped and reduced in quantity. HUNT and WRIGHT (1918) noted the clumping of pigment in dilute guinea pigs. GREMMEL (1939), in his study of the pigmentation of

[2] The name platinum was presumably given this color phase as a result of the popularity of the platinum silver color phase in the fox. The use of the same name to designate color phases in both species should not be taken as suggesting a similarity in expression of the mutant genes involved. The pelt of the platinum color phase is designated "Silverblu" by the MUTATION MINK BREEDERS ASSOCIATION.

[3] A brown mutant color phase called "Burgundy" is known in the red fox.

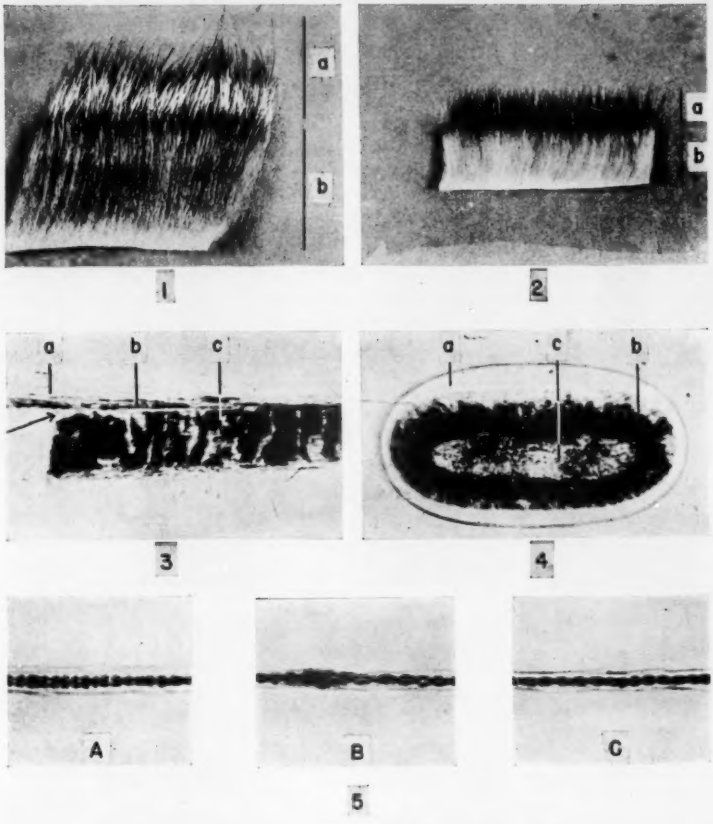


FIGURE 1.—A small section cut from the rump area of the pelt of a silver fox showing the underfur (b) and the portion of the guard hair (a) that projects beyond the underfur.

FIGURE 2.—A small section cut from the rump area of the pelt of a silver sable mink. The silver sable color phase of the mink is particularly well adapted for comparison of the guard hair (a) and the underfur (b) since the major effect of this dominant mutant gene is a general diminution of pigment production in the underfur with no effect on the guard hair except for the small number of white ones scattered over the entire body.

FIGURE 3.—Oblique section of a guard hair of the pearl fox. Arrow indicates separation of cortex (b) and medulla (c); pigment granules in the cortex oriented with long axis parallel to long axis of hair. ×370

FIGURE 4.—Cross section of a guard hair of a dark ranch-bred mink showing the cuticle (a), cortex (b), and medulla (c). ×370

FIGURE 5.—Portions from whole mounts of single hairs from the underfur of the three color phases of the mink; (A) dark, (B) platinum, and (C) pastel. ×400

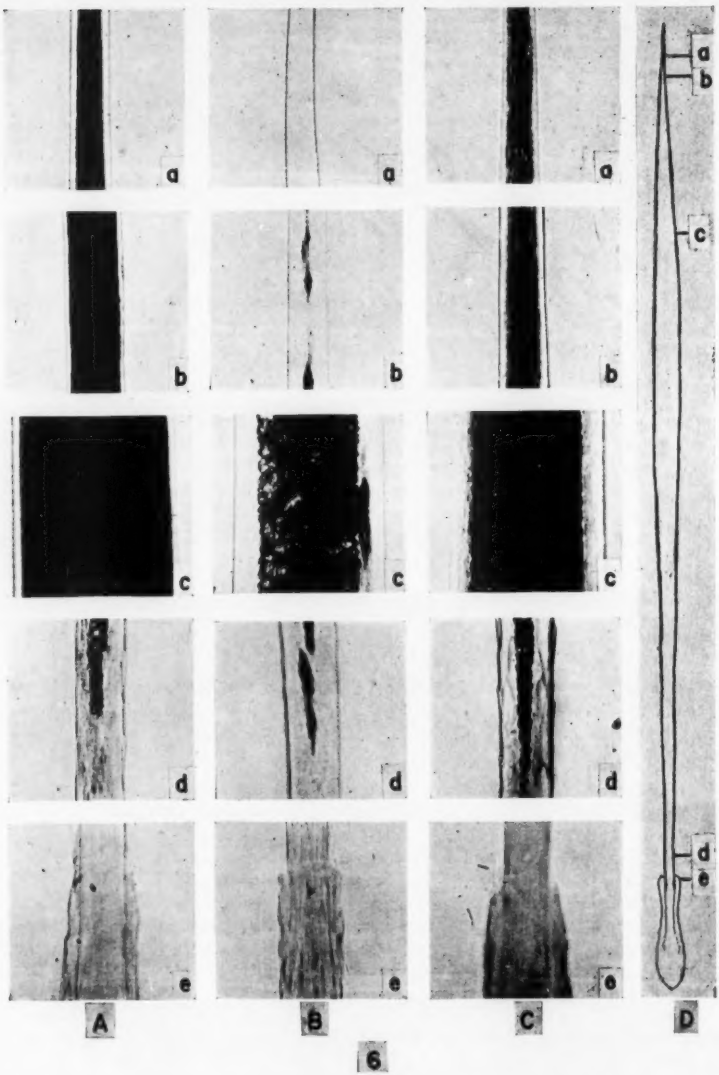


FIGURE 6.—Photon.icrographs of whole mounts of single guard hairs from three color phases of the mink; dark (A), platinum (B), and pastel (C). The approximate level at which the five portions of each hair were taken is indicated in the diagrammatic illustration (D). ×300

horse hair, found that "crowding of pigment to one side is characteristic of all dun horses." The histological technique has been used to distinguish differences in coat color by DANNEEL (1936) in the rabbit, HARMON and CASE (1941) in the guinea pig and E. S. RUSSELL (1946) in the house mouse.

The pelage of fur bearing animals consists of two rather distinct types of hairs which for purposes of classification can be designated arbitrarily as the underfur and the guard hair. The hairs composing the underfur are smaller, shorter, and more numerous (figs. 1b and 2b) than the guard hairs. These latter project beyond the underfur for one fourth to one third of their total length, the overhanging part being known collectively as the "veiling" or "covering" (figs. 1a and 2a).

Three areas are readily distinguished in the definitive hair: the medulla (fig. 4c), a central portion composed of loosely arranged cells with many intercommunicating spaces; the cortex (fig. 4b), a cylinder immediately surrounding the medulla and composed of keratinized cells; the outermost layer or cuticle (fig. 4a), composed of scales arranged in shingle fashion (figs. 5 and 6Cd) the overhanging edges toward the tip of the hair.

The young hair emerging from an active follicle is without a medulla for a short distance (fig. 6Ba and Ca), but as growth continues and the diameter increases the medulla begins to appear irregularly (figs 6Bb and Cb), becomes more continuous, and finally occupies the greater portion of the hair in the region of its greatest diameter (figs. 6Bc and Cc). As the diameter of the hair begins to regress, the medulla also begins to occupy less space until it becomes interrupted and finally disappears altogether a short distance from the root (figs. 6Ad, Bd and Cd). This series of events results in the hair having a cuticle and cortex that are continuous from tip to root, but with the distal tip and the base free of the medulla. The hairs of the underfur differ slightly from the above description. Their medulla is usually discontinuous throughout its length, the air spaces alternating in regular fashion with areas similar in appearance to the cortex (fig. 5).

Pigment granules in the cortex of the hair are oriented with their long axis parallel to the long axis of the hair, but in the medulla the granules appear to be arranged at random (fig. 3). This arrangement is characteristic of all color phases of mink and foxes, but is difficult to observe in the guard hairs of heavily pigmented color phases.

The tip of the guard hair is most diagnostic of color phase (figs. 6Aa, Ba, and Ca); consequently these observations have been limited to the distal one fourth of the guard hair. The type of pigment granule distribution in the silver color phase has been taken as the "wild type" for comparison with mutant colors in the fox since the pigment in the wild red (both red and black pigment) is arranged in a manner similar to that in the silver, and the type distribution found in the dark ranch mink as "wild type" for comparison with the mutant color phases in the mink.

Whole mounts and cross sections of representative guard hairs have been chosen for histological study because of the difficulties inherent in the study

of the underfur. The small diameter and the relative fragility of the underfur makes sectioning difficult, and the differences in pigmentation characteristic of the various color phases are less readily distinguished than in the guard hairs (Compare figs. 5 and 6).

Color of Pigment Granules

Color of the individual pigment granule is very difficult to determine. When seen in the hair (either in whole mounts or cross sections) under the microscope, the variation of a single granule from the darkest genotypes ranges from almost black to a light amber color, depending upon the amount of light admitted. Extracted pigment, either dry or in alcohol, appears dark brown in the silver, platinum and pearl color phases of the fox; the same is true of the dark and platinum mink, but the pigment from the pastel mink is distinctly lighter in color than in any of the other mink or fox color phases.

An attempt has been made to assign a color grade from RIDGEWAY'S *Color Standards and Color Nomenclature* to the pigment granules from each of the color phases considered. Color of the pigment granules does not appear to be the major cause of the differences in mutant coat color as compared to the standard coat color with the exception of the pastel mink.

Extracted granules from each of the color phases were placed on a glass slide in a thin layer, covered with a xylol solution of isobutyl methacrylate, and observed under oil immersion (objective 95×, ocular 20×). The maximum amount of light was admitted, a daylight filter being used on the lamp. Under these conditions, the granules from the silver, platinum and pearl color phases of the fox and the dark and silverblu color phases of the mink came nearest matching Sudan Brown. Granules from the pastel mink were somewhat lighter, being nearer Ochraceous-Tawny.

Materials and Methods

The number of different samples from which individual hairs have been taken for microscopical observation are shown in table 1. Most of these samples were plucked from the rump of living animals, although a few were taken from other parts of the body such as the shoulder, head and tail. All of the samples used for the photomicrographs, however, were collected from the rumps of live animals with a prime pelt (November, 1946). Guard hairs of silver, platinum and pearl foxes, and dark, platinum and pastel mink were cleaned by washing in an ether bath (described in the following section), and allowed to dry at room temperature. The hairs that were to be observed as whole mounts were placed on a glass slide and covered with a thin layer of methacrylate solution instead of being mounted in the usual manner. The low power (objective 10×, ocular 10×) of the microscope was found adequate in examining the whole mounts: all photomicrographs were taken with the high power (objective 44×, ocular 10×).

Hair is very difficult to section without tearing, and as pigment arrangement was the object of this study, a method to obtain undamaged cross sections was

of prime importance. HARDY'S (1935) method proved excellent for studying tufts of hair, but cross sections prepared in this way were usually crowded so much that one impinged upon the other, either distorting the individual section or interfering with illumination. This method is also inadequate when it is desired to cut cross sections as nearly the same thickness as possible. Further, it is important that comparable regions of hairs of comparable lengths be obtained, and this method allows little control over this operation. Since isolated and undamaged cross sections were necessary, other methods of sectioning have been investigated.

The following method, although somewhat time consuming, was found to

TABLE 1

Number of samples from which hairs have been taken for histological observations.

SPECIES	COLOR PHASE		NUMBER OF ANIMALS SAMPLED
Fox	Silver		70
	Pearl		29
	Platinum	Werth 15	43
		Cody 10	
		Norwegian 12	
		La Forrest 6	
Mink	Dark		35
	Platinum		20
	Pastel		24

give the best results. Guard hairs taken from the same samples as those used for the whole mounts and cleaned in the same way, were dipped individually into a xylol solution of isobutyl methacrylate polymer (E. I. DU PONT DE NEMOURS & CO., INC), and allowed to dry at room temperature. Drying was usually completed in one to two hours. Applications of the methacrylate solution were made until a layer of the dry material about 1 mm thick surrounded each hair. Three hairs prepared in this manner were placed together and dipped into the solution and allowed to dry overnight.

These groups of three hairs, encased in a coat of the methacrylate, were mounted in paraffin (melting point 50°–54°C) in the usual manner, and sectioned at ten micra. The paraffin ribbons were fixed on glass slides, allowed to dry overnight, and the paraffin and methacrylate removed with xylol. A thin layer of the methacrylate solution was applied to the slide in place of an ordinary cover glass.

The methacrylate coat served two purposes; it held the hair firmly and allowed it to be sectioned with a minimum of distortion, and the thickness of the methacrylate coat between each individual in the group of three hairs

allowed the proper spacing so that one section did not press against the other and either distort it or interfere with illumination. Sections cut much thinner than ten micra were apt to tear, and if much thicker, the more densely pigmented ones were too opaque to allow the arrangement of pigment to be studied in detail.

Results

The cross sections revealed a notable difference in shape of the guard hairs of the mink and foxes. The fox guard hair is approximately round in cross section throughout its length. Cross sections of mink guard hairs are oval in shape except at the tip and base which are round; the ventral side of the oval cross section is less curved than the dorsal side (fig. 4).

Mink

Dark

The guard hair of the dark mink is so heavily pigmented that the internal structure is completely obscured in views of whole mounts (fig. 6A), except that the cuticle can be seen to be pigment free. Cross sections reveal that the dark brown pigment granules are arranged singly or closely packed in groups in the cortex and medulla (fig. 8A). The non-medullated tip is more densely pigmented than any other part of the guard hair.

Platinum

The platinum (fig. 6B) shows the greatest contrast of any of the color phases when compared with the wild type (dark) distribution. The pigment granules are dark brown in color like those of the dark mink, but in longitudinal views of a guard hair the pigment gives the impression of irregular transverse bands alternating with light areas relatively free of pigment. Cross sections (fig. 8B) show that the pigment is mainly clumped in the cortex and medulla, and that most of the pigment is in the medulla, resulting in the cuticle appearing considerably thicker than in the dark mink. The non-medullated tip is free of pigment.

Pastel

Enough light passes through a whole mount of the guard hair of the pastel to reveal the cortex and medulla (fig. 6C). In cross section (fig. 8C), the pigment granules do not appear to be so closely packed as in the dark mink, and are distinctly smaller and lighter in color than in either the dark or platinum; except for the fact that the outer margin of the cortex is sometimes low in pigment, the distribution is similar to the wild type (dark).

Foxes

Silver

The denseness of the pigmentation in the silver fox obscures the internal structure of the hair as in the dark mink. The transparent pigment-free cuticle appears to be about one-third as thick as the cuticle of the mink guard hair

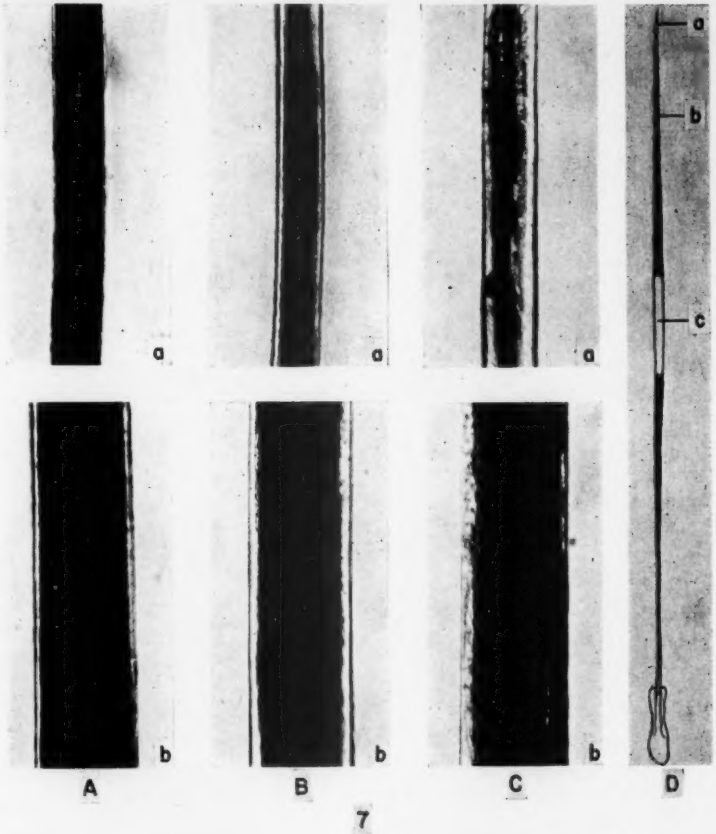
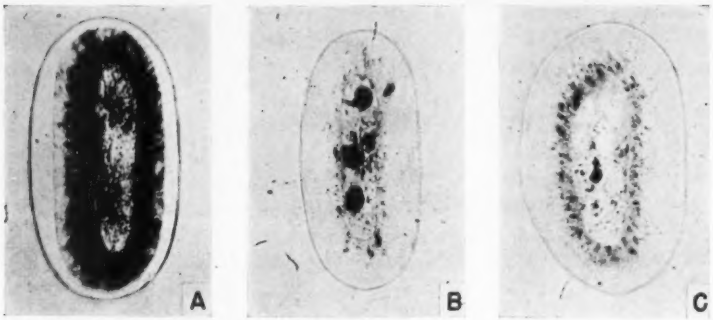


FIGURE 7.—Photomicrographs of whole mounts of single guard hairs from three color phases of the red fox; silver (A), platinum(B), and pearl (C). The approximate level at which the two portions of each hair were taken is indicated by the diagrammatic illustration (D). The "silver" bar characteristic of all color phases of the red fox is shown (c). ×300

(compare figs. 9A and 8A). A cross section (fig. 9A) reveals the numerous pigment granules oriented at random in the medulla, and a cortex packed from the inner to the outer margin with single granules and groups of granules. The most densely pigmented part of the guard hair is the non-medullated tip.

Platinum

No pigment can be seen at the extreme tip of the platinum guard hair, but pigment gradually appears (fig. 7Ba) below this area. The general sparseness of pigment allows the internal structure of the hair to be readily seen in light and medium colored individuals. In the darker platinums, only the extreme



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9

FIGURE 8.—Photomicrographs of a cross section of a guard hair of the dark (A), platinum (B), and pastel (C) color phases of the mink taken at the level indicated in the diagrammatic illustration. ×300

FIGURE 9.—Photomicrographs of a cross section of a guard hair of the silver (A), platinum (B), and pearl (C) color phases of the red fox taken at the level indicated in the illustration. ×300

tip of the guard hair is diagnostic since the platinum and silver guard hair may be similar in appearance below the area where the medulla has become continuous, or in the vicinity of a "silver" bar. The translucency of the tip of the platinum guard hair is easily seen with the unaided eye; this characteristic has been especially useful in distinguishing some of the lightest "white-marked" silver phases (COLE and SHACKELFORD) from the true platinum color phase.

Granules in the platinum guard hair are distributed in the same manner as in the silver fox, except that there are smaller numbers of granules per unit area, as can be seen in a cross section (fig. 9B). The pigment in the cortex first appears in the area adjacent to the medulla and gradually approaches the outer margin of the cortex as the diameter of the hair increases. The cuticle appears to be thicker than in the silver, but this is the result of a tendency

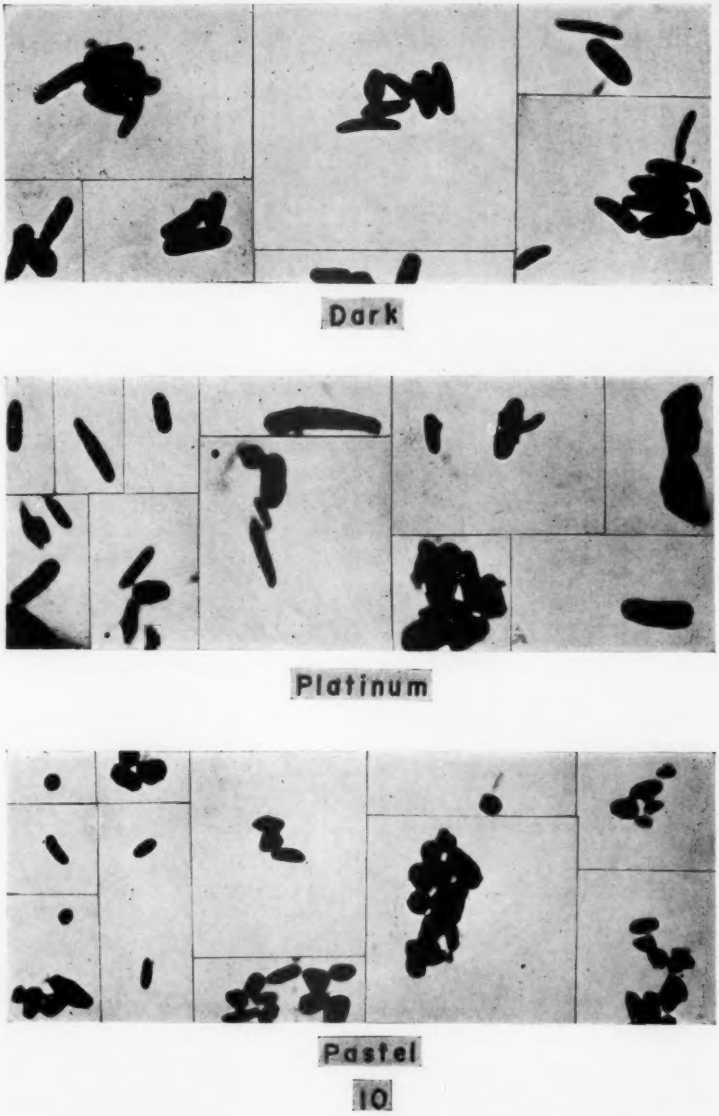

FIGURE 10.—Pigment granules extracted from the hair of three color phases of the mink. ×9,130

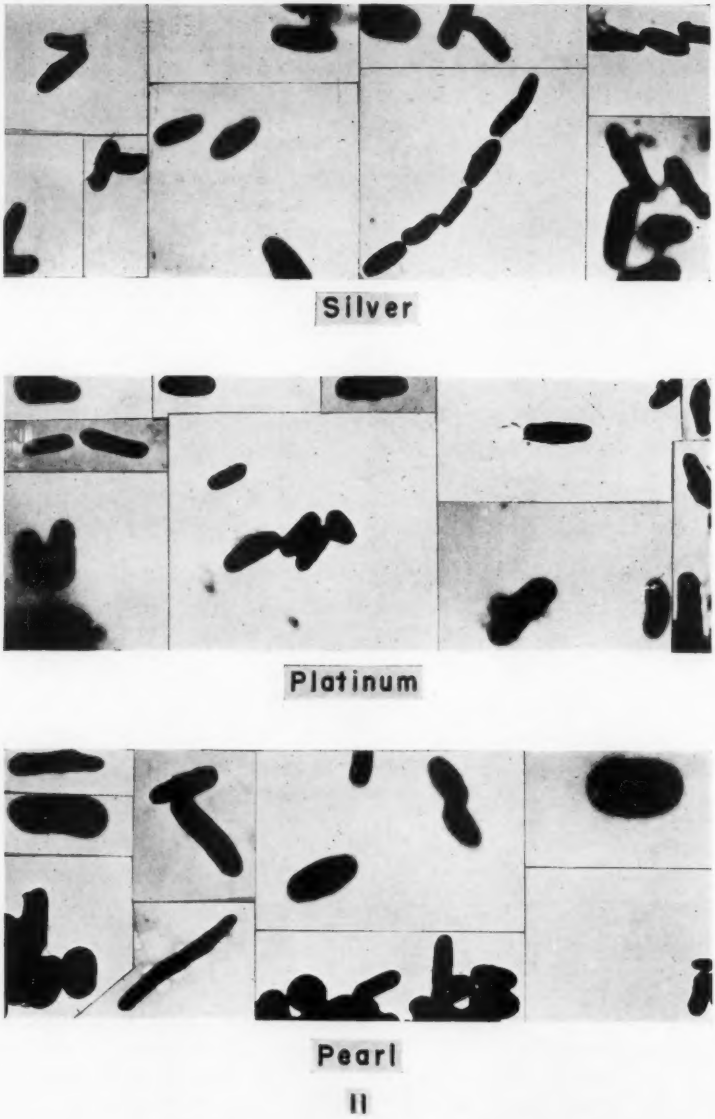

FIGURE 11.—Pigment granules extracted from the hair of three color phases of the red fox. ×9,130

for the small amount of pigment in the cortex to be arranged around the medulla. In the photomicrographs the granules appear lighter in color than in the silver as a result of more light passing through the hair section.

Pearl

The pigment clumps characteristic of this color phase appear as a series of irregular transverse bands in whole mounts (fig. 7C), making the pigment distribution in the pearl guard hair the easiest of all the color phases to distinguish from the "wild type" distribution. Clumping is most readily observed near the tip, but can be seen the entire length of the hair. As in the platinum, the cuticle appears to be thicker than in the silver guard hair.

The majority of the granules in this color phase are combined into relatively large aggregates, although a number of individual granules and small groups of granules are occasionally found (fig. 9C). Most of the clumps are in the medulla, but the majority of the pigment in the cortex is clumped and tends to occupy the area of the cortex contiguous to the medulla.

QUANTITATIVE MEASUREMENT OF MELANINS

The chemical inactivity of the melanins makes extraction difficult. EINSELE (1937) has considered the possible methods of separating melanins from the hair protein (keratin) and concluded that acid hydrolysis is the most suitable. He developed a technique which gave reliable results in the analysis of pigment production in mice of various genetic constitutions. EINSELE's method has been used in these studies.

This method depends upon the differential reaction of melanin and keratin to acid treatment; keratin is hydrolyzed by sufficiently long treatment, but melanin appears to be unaffected. E. S. RUSSELL (1939) has used this technique successfully to obtain pigment for her studies on quantitative differences in pigmentation in the sepia series of guinea pigs. She has called attention to the distinction made by GORTNER (1912) between acid-soluble and acid-insoluble melanins, and in this connection has commented as follows: "It should be noted that EINSELE's statement concerning the insolubility of mouse melanin depends on the fact that the color obtained in the acid hydrolysate is always pale yellow, showing no correlation with the amount of melanin in the hair treated. By the same reasoning the same conclusion holds for guinea pig melanins." The same results were obtained in the extraction of fox and mink melanins, the acid hydrolysate always being pale yellow regardless of the color of the hair treated.

Materials and Methods

Fur samples were obtained from living animals during the first week of May (1946) before the onset of the spring moult. A method thought to represent the different color phases adequately was the selection of two individuals that were approximately midway between the darkest and lightest in coat color characteristic of the range in their respective color phases. Fur samples from the standard silver, platinum and pearl color phases of foxes were collected

on a commercial fox farm; the samples of dark, platinum and pastel mink were obtained from animals in the University experimental herd.[4]

Although foxes are large enough to produce adequate samples in relatively limited areas of the body, the area chosen was the rump since there is greater uniformity of silvering between the various color phases on this area. The hair from an area approximately 10 cm×12 cm was removed for each sample. The small size of the mink and the shortness of the fur made it necessary to use a larger area; the fur on the back from the shoulders to the tail and well down the sides was removed for each sample. The fur samples were cut as close to the skin as possible by the use of ordinary scissors. The density and finess of the underfur, especially in the mink, made the use of electric clippers practically impossible.

Fat and dirt must be removed from the fur to circumvent two sources of error in determining the total hair weight attributable to melanin. Probably the greatest error results from the loss of granules that become entrapped in the soap ring formed inside the flask during hydrolysis, due to the action of acid on fat. Another source of error results from the increased weight of the hair due to impurities. EINSELE found that in mice dirt and fat could account for as much as 12 percent of the total weight of a hair sample.

Coarse dirt and some of the fat were removed by washing each fur sample in a 0.1 percent solution of sodium lauryl sulphate (sold commercially as "Dreft") for one hour as suggested by RUSSELL (1939). The solution was decanted and the fur rinsed three times with hot distilled water. After the last rinsing, the sample was divided into eight portions and each portion spread in a layer approximately .5 cm deep in the trays that are described below. The trays containing the wet fur were placed in a drying oven kept at 95°–105°C where they remained for four hours, then transferred to a desiccator until needed for further treatment.

EINSELE (1937) found that washing with water removed only part of the fat, and that further cleansing was necessary. He devised a bath which allowed the hair to be washed continuously with clean ether. The principle used by EINSELE has been applied in making the fat extractor for these studies.

A large glass vacuum desiccator (Scheibler) with the hole in the top was found satisfactory. The condensing coil was made of glass tubing. The hole fitted with a countersunk rubber stopper served as an entrance and exit for the coil. To insure sealing the stopper in the hole and the coil in the stopper, melted paraffin was poured over the stopper until it was flush with the surface of the collar. This procedure firmly attached the condensing coil to the top, and reduced the chance of breakage at each time the bath was opened. The top was sealed to the bowl of the desiccator by a thin coating of vaseline, and to prevent the escape of vapor due to pressure increases, two small thumbscrew type clamps were used to hold the top securely in place.

The trays for holding fur samples in the ether bath were made of copper screening (19 mesh). A rack to support the trays was fashioned of copper wire

[4] Cooperative experiments with the WISCONSIN CONSERVATION DEPARTMENT'S EXPERIMENTAL GAME AND FUR FARM, Poynette.

(No. 12), and had a capacity of four 10 cm×10 cm trays. The four posts of the rack extended 1.5 cm above the top tray, and served as a rest for the piece of copper screening which acted as a baffle in dispersing the drops of ether as they rained from the condensing coil.

The apparatus was set in a water bath on a tripod, and a 250 watt light bulb used as a source of heat. This arrangement kept the ether boiling, and the water running through the coil at tap temperature condensed it with sufficient speed to produce a continuous shower on the trays. Approximately 5 cm of ether was kept in the bottom of the desiccator; this had to be replenished each time the top was removed due to escape of vapor.

Preliminary tests indicated that the fur samples ceased to lose weight after two days in the ether bath, but to insure complete cleaning a four day treatment period was used. Each sample started at the bottom position on the rack, advanced upward by one position each day until at the end of the fourth day it was removed, the ether allowed to evaporate at room temperature, and the sample placed in the drying oven for one hour. Samples were stored in a desiccator until weighed; after three hours the fur samples ceased to lose weight, so a minimum of four hours in the desiccator was allowed before hydrolysis was begun.

Each portion of the sample from an individual, weighing 500–2000 mg in the fox and 500–1500 mg in the mink, was transferred to a 500 cc pyrex Florence flask fitted with a reflux condenser, 300 cc of 6N hydrochloric acid added, and the mixture boiled for two hours. The keratin appeared to be completely hydrolyzed after one hour, but boiling was continued for another hour to make sure all melanin granules had been liberated. After cooling, the acid solution was centrifuged at 3000 r.p.m. for 10 minutes and the liquid decanted. The residue was washed four times with distilled water and as the solution no longer gave a chloride test, the pigment was considered free of the keratin. In some of the lighter pigmented color phases, considerable difficulty was experienced in getting the melanin granules to settle out from the acid solution, even when centrifugation was continued for an indefinite time. This was overcome by diluting the solution with distilled water.

Following the fourth washing, the water was decanted and the pigment washed from the centrifuging tube into weighing bottles with 95 percent ethyl alcohol. The weighing bottles were placed in the drying oven and allowed to remain for one hour after the pigment appeared to be completely dry, then placed in a desiccator to remain until a constant weight had been attained (completed in all cases by four hours). These weights were recorded, and the percent of total weight of the hair sample represented by the melanin pigment was calculated. The final figure tabulated in table 2 is the mean of the eight portions of each sample.

Results and Discussion

Histological studies in several animals have indicated an apparent difference in the quantities of hair pigment in some of the lighter colored genotypes as compared to the darker. As early as 1904 Miss DURHAM made this observation

in her studies of dilute mice. The same observation was made in the histological studies of the guard hair of mink and foxes, but in certain color phases such as platinum in the mink and pearl in the fox the density of the individual pigment clumps could not be determined by visual inspection, so extraction was necessary to determine the relative quantities of pigment produced by the different color phases.

TABLE 2

The percent of melanin pigment in the fur of some color phases of mink and foxes.

SPECIES	COLOR PHASE		ANIMAL NUMBER	MEAN (%)	STANDARD DEVIATION	COEFFICIENT OF VARIABILITY (%)
Mink	Dark		1	4.2	.1	2
			2	4.3	.1	2
	Platinum		1	1.2	.1	8
			2	1.4	.1	7
	Pastel		1	2.1	.1	5
			2	2.4	.1	4
Fox	Silver		1	5.0	.3	6
			2	5.0	.3	6
	Platinum	Werth	1	1.9	.1	5
			2	1.8	.1	6
		LaForest	1	2.1	.2	10
			2	1.9	.1	5
	Pearl		1	3.9	.1	3
			2	3.8	.3	8

The following generalizations can be made insofar as the two individuals of each color phase can be considered as representative of their respective color phases: in mink the approximate portion of the hair weight attributable to melanin pigment is 4.3 percent in the dark, 1.3 percent in the platinum and 2.3 percent in the pastel; for foxes, 5 percent in the silver, 1.9 percent in the platinum and 3.9 percent in the pearl. The pastel and dark mink bear the same relation to each other in quantity of pigment produced as reported by DUNN and EINSELE (1938) for brown and black mice; that is, pastel produces about one-half as much pigment as does the dark mink.

EINSELE (1937) found that as a result of long boiling, melanoids were formed during hydrolysis by the action of acid on keratin, and that these substances adhered to the melanin granules. By the use of albino hair he was able to determine that the quantity of melanoids formed and their ability to adhere

to the melanin granules were independent of the quantity of the granules in the hair, and thus relatively greater errors were introduced in determining the quantity of pigment produced in the lighter than in the darker genotypes. He arrived at a correction of this error in his work with mice as follows: "If such hairs are hydrolyzed together with heavily pigmented hairs, then the combined residues may be easily centrifuged out. Thus, if one uses as adsorbing agents the granules of a weighed quantity of pigmented hair of known pigment content, then from the differences between the weight of the total residue and the calculated weight of the granules used as adsorbing agents the percentage content of such lightly pigmented hairs can be easily calculated."

This correction was not applied in determining the pigment content of the fur of the various color phases of mink and foxes because pigment free fur was not available. No overlaps were observed between the different genotypes as to quantity of pigment produced, so the uncorrected determinations were considered adequate to indicate the relative differences in pigment production. DUNN and EINSELE (1938) found uncorrected weights satisfactory in their comparisons of the pigment producing abilities in the different genotypes of mice.

MEASUREMENTS OF PIGMENT GRANULE LENGTH

Some differences of pigment granule size and shape in the pastel and dark mink are apparent from cross sections of the guard hair; in the pastel most of the granules are considerably smaller, and appear to be more spherical than rod-shaped as are most of the granules in the dark mink. This observation suggested that some of the differences in fur color in the different genotypes of mink and foxes were related to granule size, shape, or a combination of these qualities.

Using preparations of melanin granules isolated by acid hydrolysis, EINSELE (1937) found that in mice different genotypes have characteristic granule size distributions. RUSSELL (1946) was able to measure the medullary pigment granules in various genotypes of mice from cross sections of the hair, stating that "The complete outline of individual granules is clearly visible in cross sections of even the most crowded types."

An approximation of the range in size of pigment granules characteristic of each genotype was desired, and this could not be obtained with cross sections because of the orientation of the granules in the cortex (fig. 3b) and the crowded condition in both cortex and medulla (fig. 4). Further, the clumping characteristic of two color phases (platinum in mink and pearl in the fox) possibly prohibit representative measurements of the individual granules in cross sections. Hence, in these studies the melanin granules extracted by acid hydrolysis from mink and fox fur were used.

RUSSELL's (1946) observation that in dilute mice there "are large sharply defined clumps which may quite possibly be composed of the regular granules of the genotype concerned cohering to each other" applies to the present observations in mink and foxes. These clumps, however, appear to be completely broken up into their component granules by the acid treatment.

There has been considerable controversy since the first investigations of the underlying factors in hair color differences as to whether granular pigments were the only pigments involved. Several of the earlier investigators thought that diffuse pigments were present in addition to granular pigments in certain colors of hair, and at least one declared that the color of blond and red hair in humans was due solely to diffuse pigments. EINSELE (1937) has considered this question at length. He found that in the mouse "With the appearance of the least pigmentation granular pigments are always found . . . " and concluded that "Since we have been able to measure the pigment granules from hairs of all grades of pigmentation and to correlate their sizes with the visible hair colors it is unnecessary to assume the existence of diffuse pigments." No evidences of diffuse pigments were observed in mink and fox hairs, although it should be remembered that the colors here considered are comparable to the sepia series in other mammals, while the yellow series is the one involved in this controversy.

The minuteness of melanin pigment granules makes an approximation of their size and shape difficult with the ordinary light microscope, although both EINSELE (1937) and RUSSELL (1946) have accomplished this feat with mouse pigment. The great magnifying power of the electron microscope, however, makes it invaluable in a study of this kind.

Methods and Materials

An RCA model EMU-1 electromagnetic electron microscope has been used in these studies. Slides for use with the microscope must be prepared in a special manner. One way of making slides is as follows: the small screen disc (200 mesh) which acts as a support for a thin film upon which the material to be examined rests, is placed on an ordinary glass slide in the bottom of a dish of water; a film is prepared by placing a drop of parlodian on the surface of the water where it immediately spreads into a thin layer approximately .01 micra in thickness; the glass slide is raised toward the surface until the film comes to rest on the screen disc, then the slide and its contents are carefully removed and placed in a desiccator so that all moisture will be removed. The slide prepared in this special manner is placed in the microscope, and the objects to be observed are seen as silhouettes on a fluorescent screen.

Pigment granules obtained from the quantitative measurements were stored in 95 percent alcohol for use in the qualitative studies. The melanin extracted from the eight fur samples of each of the individuals representing their respective color phases was put into a single flask, and as a consequence a pigment sample taken from any one flask would contain granules produced by both animals, presumably in equal numbers. To make sure that the granules were thoroughly mixed at the time a sample was taken, the flask was shaken vigorously.

Great difficulty was experienced in getting individual granules free of their neighbors since extracted melanin granules cling together in large clumps. For use with the light microscope where the orthodox method of preparing slides can be used, a little "wet" pigment directly from the centrifuge tube diluted

with water makes a satisfactory preparation. Slides for use with the electron microscope can not be prepared in this manner, however, because the granules clump together on the parlodian film as the water evaporates.

The following method of preparing slides for use with the electron microscope was found satisfactory. About 2 cc of the stored granules were poured into a watch glass and the alcohol allowed to evaporate. As much of the resulting dry pigment as would cling to the end of a small glass rod was stirred with two or three drops of parlodian on a glass slide, the mixture taken into a pipette and dropped on the surface of the water. Spreading of the parlodian to form the film brought about two desired results: many granules were either isolated singly or in small groups in such a manner that their size and shape could be observed, and the granules appeared to be oriented with their long axis parallel to the surface of the film.

All of the unencumbered granules in a square chosen at random were observed and their lengths approximated. This process was continued until 200 granules from each of the color phases had been measured. The unit of measurement was taken as one-tenth of a side of one of the four squares of equal size on the fluorescent screen. At a magnification of 10,140 diameters, this unit was equivalent to .19 micra.

Results and Discussion

Several qualitative differences in the granules of the different genotypes can be seen from the photomicrographs shown in figures 10 and 11. In both species most of the granules are rod-shaped, the pastel mink being the only one in which appreciable numbers of round, or near round, granules occur. Further differences noted in the mink are that granules of the pastel are notably smaller in comparison with the other two genotypes, and that very large and long granules are occasionally seen in the platinum, which in other respects appears similar to those of the dark mink. In the three genotypes of the fox, such differences as exist are less noticeable than in the mink, the exception being the notably greater range in size of the granules in the pearl fox as compared with either silver or platinum.

Length is a component of size that can be directly approximated and therefore has been used as a measure of quantitative differences between the different genotypes. The frequency distribution of granule length in each of the different genotypes is shown in figure 12. Granules smaller than one unit (.19 micra) were included in the first class, but in only one genotype, pastel in mink, could this grouping have resulted in appreciable errors since there are relatively few granules in this class. Granules approximating .19 micra in greatest diameter or smaller are for all practical purposes round in shape.

The dark genotype in mink and the silver genotype in the fox have been taken as the standard with which to compare the mutant genotypes of their respective species. Two features of the granule size distributions have been tested against their respective standards; the difference in the means of the two distributions ("t" test) and a comparison of the class frequencies (χ^2 test). The results of these calculations are tabulated in tables 3 and 4.

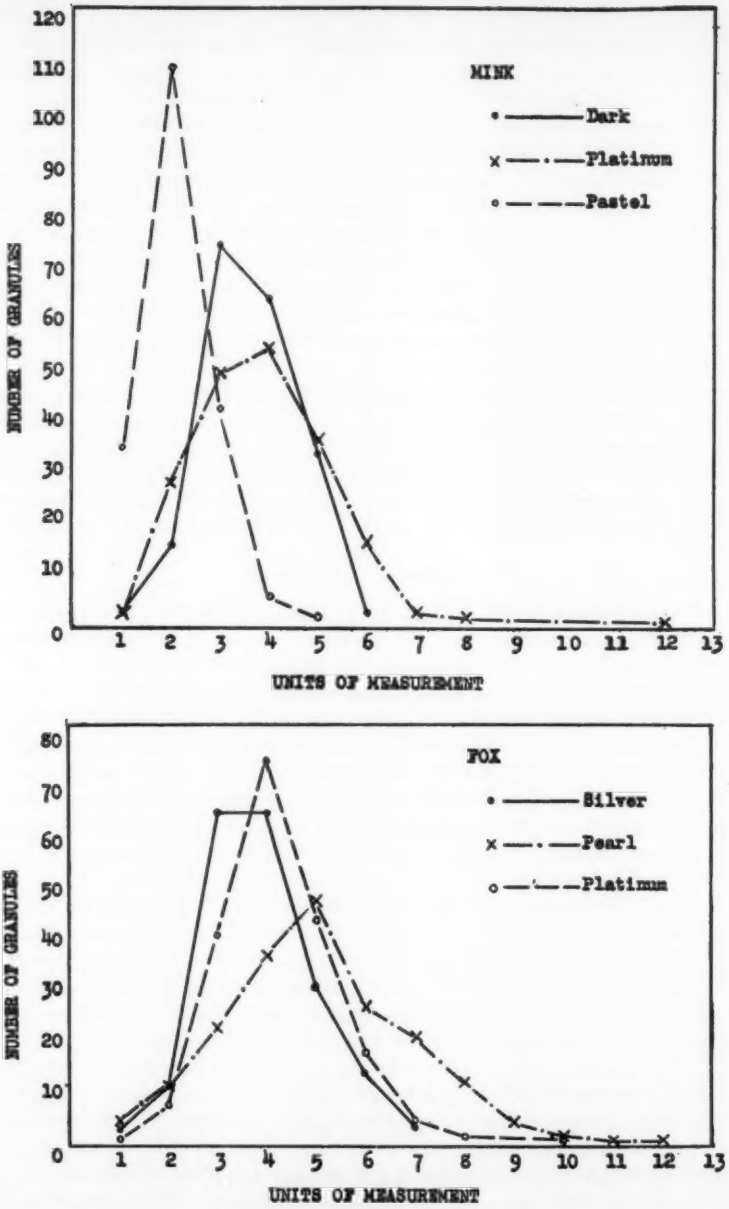

FIGURE 12.—Frequency distributions of the length of random samples of 200 pigment granules extracted from the hair of each of the color phases of the mink and fox indicated. 1 unit = .19 micra.

Foxes

Granules of the silver fox range from less than .19 micra to 1.33 micra in length with a mean length of .74 micra. In the platinum the range is from less than .19 micra to 1.90 micra with a mean length of .82 micra. The means of the two distributions differ significantly ($P<.01$), and the class frequencies are on the border line of significance ($P\cong.04$).

TABLE 3

Lengths of melanin pigment granules extracted from several color phases of the fox and mink.

SPECIES	COLOR PHASE	NUMBER OF GRANULES	MEAN (MICRA)	STANDARD DEVIATION
Fox	Silver	200	.74	.21
	Platinum	200	.82	.23
	Pearl	200	.97	.36
Mink	Dark	200	.68	.38
	Platinum	200	.74	.32
	Pastel	200	.40	.15

TABLE 4

Comparisons of the distributions of melanin pigment granule lengths of mutant color phases of mink and foxes to their respective "standards."

SPECIES	COLOR PHASES COMPARED	MEAN DIFFERENCES		CLASS FREQUENCY DIFFERENCES	
		"t" VALUE	PROB.	χ^2 VALUE	PROB.
Fox	Silver—Pearl	7.93	<.01	69.30	<.01
	Silver—Platinum	3.00	<.01	11.94	≅.04
Mink	Dark—Platinum	2.06	≅.04	15.37	<.01
	Dark—Pastel	9.60	<.01	191.07	<.01

The range in granule length in the pearl genotype is from less than .19 micra to 2.28 micra with a mean length of .97 micra. The means of the two distributions differ significantly ($P<.01$) as well as the class frequencies ($P<.01$).

Mink

The granules of the dark mink range in length from less than .19 micra to 1.14 micra with a mean length of .68 micra, and in the platinum genotype up to 2.28 micra with a mean length of .74 micra. It is questionable whether the differences between the means of these distributions are distinct enough ($P\cong.04$) to consider granule length a distinguishing feature. The size distribution of the granules of the platinum, however, differs significantly from that of the dark ($P<.01$).

Granules of the pastel mink range in length from less than .19 micra to .95 micra with a mean length of .40 micra. The pastel and the dark granule length distributions overlap but the means of the two distributions differ significantly ($P < .01$) as do the class frequencies of these distributions. The relation of the dark and pastel mink in regard to size of pigment granule approximates the results of DUNN and EINSELE's (1938) investigations with black and brown mice, that is, "The chief effect of the mutant change from B to b is thus to decrease the size of the units in which melanin is deposited."

Some of these observations are strikingly similar to RUSSELL's (1946) observations on pigment granule size in mice. She found the narrowest range in chocolate mice, this being similar to the pastel mink; the widest range was in dilute mice which is comparable to the pearl and platinum mutations. The granule lengths in her dilute mice, which are presumably comparable to platinum in the mink and pearl in the fox, tended to be skewed toward large size. This is also true in both pearl and platinum (fig. 12).

SUMMARY

An attempt has been made to characterize some of the quantitative and qualitative factors of pigmentation involved in coat color differences in several mutants of the red fox (*Vulpes vulpes* L. = *V. fulvus* species) and the mink (*Mustela vison* Peale and Beauvois). Three methods of approach have been followed: (1) histological aspects of guard hair pigmentation, (2) quantity of melanin pigment produced as determined by weight, and (3) measurements of pigment granule lengths. In the fox, the ranch-bred silver color phase has been taken as the standard to which the platinum and pearl mutants have been compared; for the mink, the standard for comparison of the platinum and pastel mutant color phases has been the ranch-bred dark.

Mink. The major effects of the platinum mutation on coat color appear to be the clumping of the pigment granules and the reduction in the quantity of melanin pigment to approximately one-third as compared to the dark mink. In both color phases the granules are dark brown in color. The granules of the platinum are more variable in length and shape than in the dark, and the frequency distribution curve of granule length is skewed toward larger size.

Pigment granules of the pastel as compared to the dark are notably smaller in size and lighter in color; greater numbers are round in shape, and the variation in length is less. The distribution of the pigment in the hair is similar to that of the dark mink.

Foxes. The major difference in the coat color of the platinum mutant as compared to the silver appears to result from a reduction in the number of pigment granules per unit area in the hair; the platinum has about 40 percent as much pigment by weight as the silver. The pigment granules are dark brown in both color phases and are the same shape. The mean length of granule in the platinum is significantly greater than in the silver, and the difference in frequency distribution between the two color phases is on the border line of statistical significance.

The pearl mutation appears to produce most of its effect on coat color by a

clumping, and reduction in quantity of the melanin pigment to approximately 80 percent of that characteristic of the silver. The granules of the pearl and silver are the same in color, but in the former the granule length and shape is more variable, and the frequency distribution curve of granule length is skewed toward the larger size.

These investigations of the nature of coat color differences in the various color phases of mink and foxes closely parallel the observations of earlier investigators working with the mouse in so far as the qualities considered are comparable and the probable homology of the different mutants of the three species obtain. The granular clumping characteristic of the dilute mutation in mice is also characteristic of the platinum mink and pearl fox; the dilute mouse, the platinum mink and the pearl fox have a greater variability in granule length than their respective standards of comparison, and the frequency distribution curves of granule length are skewed toward the larger size in all cases; in fact, the frequency distribution curve of granule length of the platinum mink and the pearl fox appear more similar to each other than either one does to the other color phases within its own species. The pastel mutation in the mink and the brown mutation in the mouse appear to be determined by a similar pattern in granule size. These color phases have the least variability in granule length of any of those investigated in their respective species. The pastel mink produces about one-half as much pigment by weight as the dark, which is comparable to the pigment producing abilities of brown mice.

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LITERATURE CITED

ASHBROOK, FRANK G., 1937 The breeding of fur animals. Yearb. U. S. Dept. Agric.: 1379–1935.

BOWNESS, E. RENDLE, 1944 The pearl platinum fox. Amer. Fur Breeder **17**: (5) 38, 40, 42, 44; (6) 42–44; (7) 34–35.

CASTLE, W. E., and LARRY MOORE, 1946 Mutations of mink under domestication. J. Hered. **37**: 137–145.

COLE, LEON J. and RICHARD M. SHACKELFORD, 1943 White spotting in the fox. Amer. Nat. **77**: 289–321.

DANNEEL, R., 1936 Die Färbung unserer Kaninchenrassen und ihre histogenetischen Grundlagen. Z. I. A. V. **71**: 231–264.

DEAKIN, ALAN, 1942 Two different strains of pearl platinums. Canad. Sil. Fox and Fur **8** (11): 35–38.

DUNN, L. C. and W. EINSELE, 1938 Studies of multiple allelomorphic series in the house mouse. IV. Quantitative comparisons of melanins from members of the albino series. J. Genet. **36**: 145–152.

DURHAM, F. M., 1904 On the presence of tyrosinase in the skin of some pigmented vertebrates. Proc. Roy. Soc. **74:** 310–313.

1908 Experiments on the coat color in mice. Rep. Evolut. Comm. Roy. Soc. **4:** 41–53.

EINSELE, W., 1937 Studies of multiple allelomorphic series in the house mouse. II. Methods for the quantitative estimation of melanin. J. Genet. **34:** 1–18.

GORTNER, R. A., 1911 Studies on melanin. J. Biol. Chem. **10:** 113–122.

1911 On melanin. Biochem. Bull. **1:** 207–215.

1912 On two different types of melanins. Proc. Soc. Exp. Biol. **9:** 120–121.

GREMMEL, F., 1939 Coat colors in horses. J. Hered. **30:** 437–445.

HARDY, J. I., 1935 A practical laboratory method of making thin cross sections of fibers. Circ. U. S. Dept. Agric. **378:** 1–10.

HARMON, M. and A. A. CASE, 1941 Genetic aspects of pigment production in the guinea pig. Genetics **26:** 474–487.

HUNT, H. R. and SEWALL WRIGHT, 1918 Pigmentation in guinea pig hair. J. Hered. **9:** 178–181.

JOHANSSON, IVAR, 1947 The inheritance of the platinum and white face characters in the fox. Hereditas **33:** 152–174.

KELLOGG, C. E., 1941 Inheritance of degree of silvering in foxes. Wildlife Leaflet 178, Fish & Wild-Life Service, U. S. Dept. of Interior.

MOHR, OTTO L., and PER TUFF, 1939 The Norwegian platinum fox. J. Hered. **30:** 227–234.

RIDGEWAY, R., 1912 Color standards and color nomenclature. Distributed by A. Hoen and Co.

RUSSELL, E. S., 1939 A quantitative study of genic effects on guinea pig coat colors. Genetics **24:** 332–355.

1946 A quantitative histological study of the pigment found in the coat color mutants of the house mouse. I. Variable attributes of the pigment granules. Genetics **31:** 327–346.

SHACKELFORD, M., 1941 Mutations in mink. Trans. Wis. Acad. Sci. Arts Lett., **34:** 45.

SHACKELFORD, R. M. and L. J. COLE, 1947 "Screw Neck" in the pastel color phase of ranch-bred mink. J. Hered. **38:** 203–209.

SMITH, S. E., C. H. WHITAKER, LEO F. DAVIDS, and PERCY V. NOBLE, 1941 The inheritance of three coat color mutations in ranch-raised mink. J. Hered. **32:** 173–176.

TUFF, PER, 1938 Nedarvningsforhold hos reven. Norsk Veterinear-Tidsskrift Nr. **4:** 193–207.

WRIGHT, S., 1942 The physiological genetics of coat color of the guinea pig. Biol. Symposia **6:** 337–355.

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